FROM THE UNIVERSITY INSTITUTE OF FORENSIC MEDICINE, HELSINKI

# THE EFFECT OF SOMATOTROPIN AND THYROTROPIN ON GRANULATION TISSUE

## A TISSUE ANALYTIC STUDY

BY

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HELSINKI 1956

HELSINKI 1956
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# INTRODUCTION

Numerous experimental and clinical investigations have shown clearly that the endocrine system plays a considerable part in the physiology and pathology of the mesenchyma and its derivatives. The various effects of the parathyroid and thyroid on bone structure are known of old. In recent years, e.g. cortisone and somatotropin have been fairly intensely studied from this point of view. The former has been found to inhibit and the latter to promote the growth of granulation tissue. Although thyrotropin is known to increase the amount of acid mucopolysaccharides in the mesenchyma, especially hyaluronic acid, its effect on granulation tissue is practically unknown. Some papers have been published on changes of the orbital mesenchyma in exophthalmic goitre, but this is no granulation tissue proper.

The present investigation followed on the caseinate amyloidosis studies effected at the Institute of Forensic Medicine (Uotila, Peräsalo & Vapaavuori 1955, Peräsalo, Uotila & Virkkula 1956). It was found in those studies that thyrotropin increased caseinate amyloidosis whereas somatotropin (STH) either reduced or enhanced the effect of thyrotropin, depending on the dosage. This called for a separate study of the effects of thyrotropin and somatotropin and their combined effect on granulation tissue.

A drawback connected with previous studies of granulation tissue has been their macroscopical as opposed to quantitative character. The result was an incomplete idea of which of the mesenchymal components, the cells, fibrils or ground substance, was

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This work has been aided by an institutional grant. (DRG 290 and DRG 290 AT) from the Damon Runyon Memorial Fund, New York, to the Institute of Forensic Medicine, and by the Sigrid Juselius Foundation, Helsinki.

The author is indebted to Nordiska Hormonlaboratoriet Ab, Malmö, Sweden, for the hormones which were kindly placed at his disposal.

involved in the hormonal effect. As a tissue analytic, line sampling method has been developed at the Institute of Forensic Medicine (Uotila 1940, Uotila & Kannas 1952) it seemed natural to utilize it in the present study.

## EARLIER INVESTIGATIONS

*Somatotropin.* — Since Li (1945) managed to isolate pure growth-promoting hormone from the anterior pituitary, it has proved to consist of albumen with a molecular weight of 46,800 and a neutral point at pH 6.85. Some 340 aminoacids could be distinguished in it; 13 per cent was glutaminic acid.

In contrast to the other hypophyseal hormones, STH has no specific object organ; its effect is considered to embrace all the organs, especially those of mesenchymal origin. STH has gained an important position in experimental pathology. Selye (1952) has shown, for instance, that large doses of STH given to experimental animals for prolonged periods are followed by changes indistinguishable from those manifested in nephrosclerosis, rheumatic nodules, myocarditis and periarteritis nodosa. The adrenals, however, play a decisive role in these changes, as no changes occur in adrenalectomized animals. Selye believes that the STH either stimulates the adrenals to secrete so-called prophlogistic steroids, or that the hormone sensibilizes the tissues. Venning & al. (1954) have shown that STH increases the secretion of the adrenal hormones producing sodium retention in healthy persons.

Reduced formation of granulation tissue has been observed in hypophysectomized rats. Taubenhaus & Amromin (1950), studying subcutaneous, turpentine-induced abscesses in rats, found that injections of STH clearly increased the proliferation of fibroblasts and the formation of collagen fibrils.

This is along the same lines as Majarakis's (1946) investigations in which it was found that somatotropin preparations increased the tensile strength of wounds.

The stimulating effect of somatotropin on connective tissue is observable also in granulation tissue which is simultaneously subjected to the inhibitory effect of cortisone (Taubenhaus, Taylor & Morton 1952). The structure of such granulation tissue surrounding a turpentine abscess showed normal organization and morphology.

Scow (1951) has shown accelerated collagen formation in the skin and muscles of thyroidectomized rats given an STH preparation for 100 days.

*Thyrotropin.* — In addition to increasing the activity of the thyroid gland, the thyroid-stimulating hormone of the adenohypophysis also affects the connective tissue directly (Asboe-Hansen & Iversen 1951).

Exophthalmus can be produced in guinea-pigs by injecting thyrotropic hormone. The histologic changes in the retrobulbar tissue manifested in such experimental exohthalmus are highly similar to the changes encountered in the progressive or malignant exophthalmus of man (Smelser 1937): marked oedematous swelling of the extraocular muscles and considerably enlarged connective tissue volume. Microscopic study reveals infiltration of lymphocytes and signs of connective tissue proliferation (Naffziger 1952).

In histochemical studies, Asboe-Hansen & Iversen (1951) showed accumulation of mast cells and gathering of mucopolysaccharides, especially hyaluronic acid, in the retrobulbar tissue of guineapigs made exophthalmic by means of thyrotropin. In addition, these authors used histochemical methods to observe the hexosamine content in the retrobulbar tissue with the aid of hydrolysis; they found that it had increased. This also suggests that mucopolysaccharides gathered in the retrobulbar tissue.

In addition, it has been shown by injecting thyrotropin into guinea-pigs that the normal fat deposits of the muscles, liver and kidneys are decreased. The same investigations revealed hyperlipemia, and circulating leucocytes that were found to contain fat droplets phagocytosed by them (Dobyns 1946, Asboe-Hansen & Iversen 1951).

The accumulation of mucopolysaccharides found in experimental exophthalmus is most marked in the retrobulbar tissue but has also been noted in the peripheral muscles and in the perirenal, peritesticular and axillary tissue (Asboe-Hansen & Iversen 1951).

The changes occur in both intact and thyroidectomized animals, but are greater with the latter. In other words, tissue reactions occur as a direct result of the thyrotropin effect on the tissues.

Thyroxin inhibits the stimulating effect of thyrotropin on the thyroid gland and the exophthalmus-producing effect (Smelser

1938) as it does the fat-mobilizing effect of thyrotropin (Iversen & Asboe-Hansen 1952).

According to these investigations, the effect of thyrotropin on connective tissue seems somewhat contrary to that of cortisone. Cortisone produces a clearly inhibitory effect on the various elements of the connective tissue, both cells and ground substance, while thyrotropin exerts a certain stimulating effect. In addition to the changes occurring in the ground substance, principally increase in hyaluronic acid, there is an increase of mast cells in the connective tissue.

This phenomenon has also been shown by spreading experiments, subcutaneously injecting with hyaluronidase thyroidectomized rabbits (Iversen & Asboe-Hansen 1953). After a 10-day thyrotropin treatment, the spreading effect was found, by means of hyaluronidase, to have increased greatly. This was attributed to the increased amount of hyaluronate in the dermal connective tissue. In such a case there is more substance available for hyaluronidase to affect, which accounts for the notable difference in spreading observable in normal skin and in skin treated with thyrotropin.

The same phenomenon is found in the skin of myxoedematous persons; compared with normal skin, the spreading of hyaluronidase shows a clear increase (Asboe-Hansen 1951).

## MATERIAL

White male rats (Wistar), aged 2 ½—3 months, were used in all the investigations. The weights of the experimental animals at the beginning of the test varied from 110—200 g, and they all gained during the experiment. While the experiment lasted the animals were given food identical both quantitatively and qualitatively, which may be of importance as proteins have been found to affect the granulation tissue and healing of wounds (Sandblom 1944). Apart from the experimental animals used in the preliminary tests and to develop the methods, the rats used in the experiments totalled 100.

The series was divided into four groups of 25 animals each. Group I comparised the control animals which instead of hormone preparations received intramuscular injections of physiological saline solution; the animals of Group II were given 25 tibia units

of somatotropin preparation (Somacton, Nordiska Hormonlaboratoriet AB) daily by intramuscular injection; Group III was injected with a thyrotropin preparation (Actyron, Nordiska Hormonlaboratoriet AB), 0.1 USP units per day. In Group IV both somatotropin and thyrotropin were given simultaneously on the same dosage schedule as above.

In each series the experimental period for 12 animals was 5 days and for 13 animals 14 days. They were then sacrificed.

## METHODS

The same general method was applied in all the tests and series. To produce the granulation tissue, the cotton plug method suggested by Mayer was used; the same method was also used by Stebbins & Stoerk (1955). The cotton plug is placed under the rat's skin, in the extremely loose subcutaneous connective tissue between the skin and the fascia. As a result of the irritation caused by the cotton plug granulation tissue develops in the surrounding connective tissue, fills the intervals between the gauze fibres and surrounds the plug. At the end of the experiment it is fairly easy to remove the resulting granulation tissue with a fair degree of accuracy, together with the cotton plug, and to weigh it. Preliminary tests showed it to be technically easier to prepare and weigh the cotton plug granuloma than the granulation tissue produced by croton oil and the »granuloma pouch» technique applied by Selye (Selye 1953, 1955).

The operations were made under ether anaesthesia, observing the usual meticulous sterility. The cotton plugs were made by punching out bits of gauze exactly equal in size, diameter 10 mm, and sterilizing them in a hot air sterilizer.

Hair was removed from the cranial and dorsal parts of the back of the experimental animals, using scissors and an electric razor. This method was preferred to shaving as it causes no skin irritation whatever.

For the tensile strength test of the dermal wound it was decided to make the wound along the median line, some 2 ½ cm caudally from the tips of the shoulder blades, a site that could be identified in all the animals and thus giving an identical wound location for all (Fig. 1). It has been found that the tensile strength of the skin

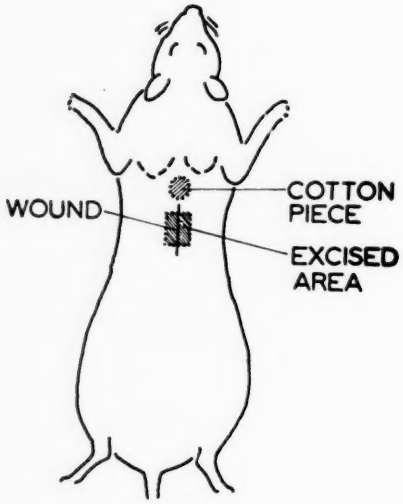



Fig. 1.

of experimental animals varies considerably in different parts of the body (Wilson 1925, Sandblolm 1944).

After cleaning and drying the skin, an approx. 3 cm long wound was made in the above location with a sharp knife held exactly perpendicularly to the skin. The incision extended into the subcutaneous loose connective tissue, splitting the cutis muscle (panniculus carnosus). Lifting the edges of the wound with forceps, the skin was cut with scissors from the muscular fascia along the loose connective tissue over an area of approx. 3 sq. cm, starting from the cranial corner of the wound and working towards the tips of the shoulder-blades. The gauze was inserted into this »skin bag» immediately caudally from the tips of the shoulder-blades; hence the gauze lay identically in the different animals. The gauze was dry when placed in position, and was therefore easy to place without creasing; its location is shown in Fig. 1.

Bleeding from the edges of the wound, which sometimes occurred during the operation, always ceased spontaneously. The wound was closed with a continuous suture, using No. 00 black silk. It has been shown (Jenkins 1937) that interrupted sutures give the firmest wound, but continuous suture reduces the potential variations and permits the most uniform closure possible of the wound (Sandblom 1944). The stiches were made 5 mm apart, 3 mm from

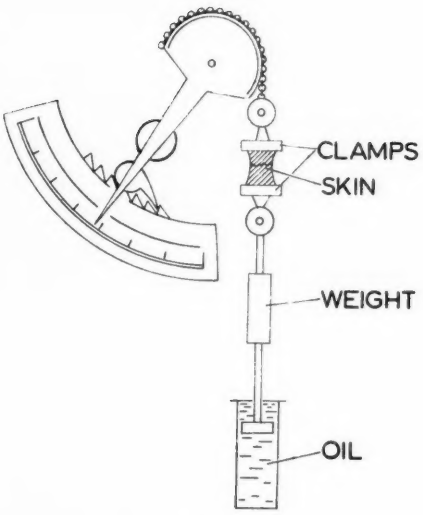



Fig. 2.

the adges of the wound, and penetrated all the split layers, including the cutaneous muscle. The wound was protected with a sterile dry bandage. A total of 3 wound infections occurred in the series.

*Determination of Tensile Strength.* — For determining the tensile strength we used a method (Howes, Sooy & Harvey 1929) in which the wound is excised and the force that causes the healing wound to be torn is measured. According to their investigations, different experimental animals show a considerable tensile strength of the wound as soon as after 5 days, when it has reached one-third of its maximum. For this reason, 5 days was selected as the experimental period for investigating tensile strength in all the series. The same period was also used by Sandblom.

For the test a square (see Fig. 1), approx. $2 \times 2$ cm, was excised from the central part of the wound. From this sample the subcutaneous connective tissue was removed carefully, and with the aid of the punch an exactly 10 mm long section of the wound, from the part that had healed best, was measured and cut off. It was placed in a container with physiological saline solution to prevent it from frying. The specimens were taken immediately to the State Materials Testing Institute.

Tensile strength was determined by H. Baer's tensiometer,

commonly used to measure the tensile strength of various textile industry fibres. The technique employed was that reported by Sandblom (1944), except that the width of the tested section of the wound was 1 cm in all the tests instead of the 1.5 cm used by Sandblom. Fig. 2 shows schematically the tensiometer and the attachment of the skin specimen to it. The wound was closely observed during the test. If the wound failed to tear suddenly and started to part from either edge, the value was not recorded; obviously, incorrect technique in making the wound or failure to place it exactly at right angles between the tensiometer clamps was involved. The tables show the number of discarded specimens.

*Histological Method.* — After the experimental animals had been sacrificed, the piece of gauze placed in the subcutaneously loose connective tissue and the granulation tissue that had developed in and around the mesh of the material was cut off in one piece from its environment and weighed immediately. After weighing, the granuloma obtained from every second experimental animal (even numbers) was divided into two parts, of which one was fixed in 10 per cent formalin and the other in »Zenker's» solution.

The microscopic preparations, after being embedded in paraffin wax, were cut into thickness of 4 $\mu$ so that every preparation gave longitudinal sections as shown in Fig. 3. (80 $\times$) The following staining methods were used on the preparations: Routine haematoxylin and eosin, Pas-staining, Pentachrome II staining (Mocat 1955).

With Pentachrome II staining the nuclei are black, the cytoplasm yellow to orange-yellow, collagen and some reticular fibres dark red, ground substance and some reticular fibres blue to bluish green. Fibrinoid stains orange-yellow. Hence the various connective tissue components can be fairly well distinguished by this staining method.

The changes of the various granulation tissue components in the different groups were determined by the »line sampling» method developed by Uotila & Kannas. The method has been applied in several investigations of the histological changes in the thyroid gland (Tala 1952, Lamberg 1953, Wahlberg 1955), and it has been described in Eränkö's work »Quantitative methods in histology» (1955). For the details and the theoretical basis of the method, reference is made to the above papers.

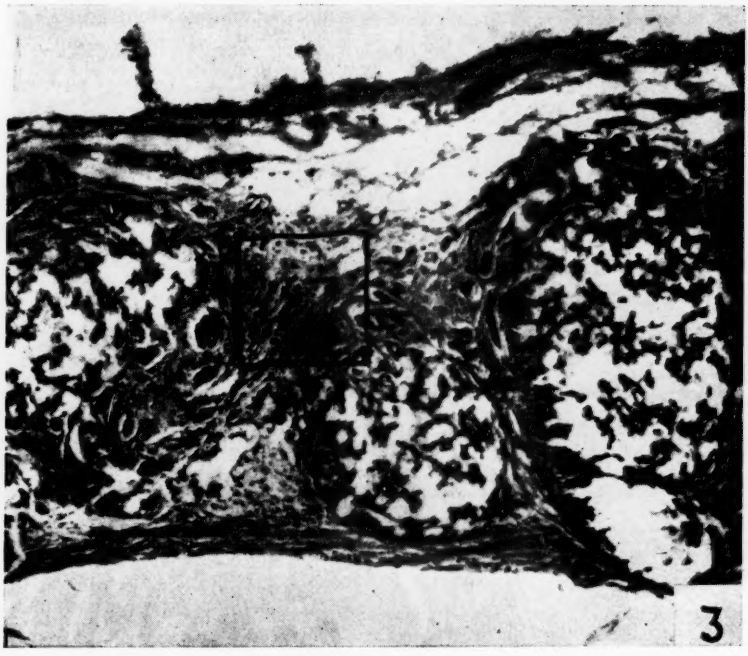



Fig. 3.

The spot shown in Fig. 3 was selected from the microscopic preparations to serve as the basis of the calculations as this spot was found to be intact and uniform in all the preparations. The calculation was made from ordinary haematoxylin and eosin stainings.

Linear determination of the various granulation tissue components was not effected from a picture projected on paper but by means of Leitz's integration ocular. This ocular has in the field of vision a hair cross moving along a hair line. The cross can be moved by winding 6 different screws. After the cross has been moved over the length of the line in the field of vision the graduation scale of each screw shows the distances the hair cross covered as a result of winding the various screws. The scale is divided into a hundred parts; hence the readings indicate per cent.

The magnification selected was 230×. With this enlargement the granulation tissue components can still be distinguished with a high degree of accuracy, e.g. the connective tissue cells from

inflammatory cells. The components whose relative volume variations were determined in the present work were the homogeneous, structureless intermediate substance (ground substance), the connective tissue cells (fibrocytes), the giant cells, the inflammatory cells, the blood vessels, the fibrils.

*Statistical Methods.* — For the weight and tensile strength of the cotton plug and the surrounding granulation tissue, the usual mean value and deviation calculations were carried out. The results are given in the tables, where

$n$ = number of observations
$x$ = mean value
$s$ = standard deviation
$\varepsilon$ = standard error of the mean.

The statistical significance of the mean values was studied by variance analysis. The results for each of the three quantities are also given in the tables, in accordance with the normal way of presenting the variance analysis, with

$v^2$ = variance quotient or the testing variable employed.
$P$ = probability that mere chance might have occasioned the observed difference in mean values.

Square brackets / / indicate cases in which the variance quotient was computed as an inverted value. The differences for which $P < 0.05$ can be considered statistically significant.

The various connective tissue components whose relative volume was computed by the »line sampling» method were studied statistically by computing first the mean value of the two determinations made per preparation (animal) and by levelling it so that the sum was exactly 100. Compiled in the tables are the subsequently computed group means and the associated deviation calculations. The latter are based on pooling the variations within the groups, which gives satisfactorily accurate deviation figures. To facilitate comparisons, the standard error in the difference between two means is ready computed. If the ratio between the difference of two means and its standard error in this material is 2.12 or more, the difference can be considered statistically significant.

## RESULTS

*Amount of Granulation Tissue in the Different Groups.* — To estimate the amount of granulation tissue in each group, some of the experimental animals were decapitated after 5 days and others after 14 days. The 5-day test of the control group comprised 15 animals and the 14-day test 10 animals; in the remaining groups — those that had been given hormones — the figures were 12 and 13 animals, respectively.

The variations in the formation of granulation tissue are shown in Table 1.

TABLE 1

| Group | Number of Observations | Mean | Standard Deviation | Standard Error of the Mean |
|---|---|---|---|---|
| *Weight of granulation tissue, 5 days* | | | | |
| Control ..... | 15 | 0.153 | 0.007 | 0.002 |
| Somacton ..... | 12 | 0.197 | 0.052 | 0.015 |
| Actyron ..... | 11 | 0.220 | 0.052 | 0.016 |
| Somacton + Actyron . | 12 | 0.212 | 0.056 | 0.016 |
| *Weight of granulation tissue, 14 days* | | | | |
| Control ..... | 7 | 0.123 | 0.023 | 0.009 |
| Somacton ..... | 13 | 0.105 | 0.009 | 0.002 |
| Actyron ..... | 13 | 0.122 | 0.008 | 0.002 |
| Somacton + Actyron . | 13 | 0.125 | 0.009 | 0.002 |

The statistical significance of the mean was studied by variance analysis, and the results for each three quantities are given in Table 2.

A reviev of the results on the basis of the analysis shows that in the 5-day test the weights of the granulomas caused by the cotton plug differ significantly in the different groups. This is due only to the fact that the mean of the control group is smaller than that of the hormone groups. The values of the groups treated with different hormones show no statistically significant differences.

TABLE 2

ANALYSIS OF VARIANCE

| Source of Variation | Sum of Squares | Degrees of Freedom | Variance | Variance Ratio | P |
|---|---|---|---|---|---|
| *Weight of granulation tissue (x 100), 5 days* | | | | | |
| Differences betweengroups . | 371 | 3 | 124 | 5.64 | 0.01 |
| Of these: C contra S, A and SA ..... | 338 | 1 | 338 | 15.36 | 0.001 |
| S and A contra SA ..... | 2 | 1 | 2 | (11.00) | >0.1 |
| S contra A ..... | 31 | 1 | 31 | 1.41 | 0.1 |
| Residual ..... | 1 022 | 46 | 22 | . | . |
| Total ..... | 1 393 | 49 | . | . | . |
| *Weight of granulation tissue (x 100), 14 days* | | | | | |
| Differences between groups | 34 | 3 | 11 | 7.86 | 0.001 |
| Of these: C contra S, A and SA ..... | 2 | 1 | 2 | 1.43 | >0.1 |
| S and A contra SA .... | 13 | 1 | 13 | 9.29 | 0.01 |
| S contra A ..... | 19 | 1 | 19 | 13.57 | 0.001 |
| Residual ..... | 59 | 42 | 1.40 | . | . |
| Total ..... | 93 | 45 | . | . | . |

In the 14-day test the groups also differ significantly. In the control group the mean of the weights of granulation tissue has been levelled and shows no essential deviation from the mean level of the hormone groups. Group IV (Somacton Actyron) differs significantly from the mean level of Groups II and III, and Somacton, finally, gives a significantly lower mean value than Actyron.

*It seems, therefore, that to begin with* (during 5 days) *both somatotropin and thyrotropin increase the amount of granulation tissue, whether administered alone or in combination. However, no differences are observable indicative of a higher potency in one or other of the hormones in this respect.*

*Later* (during 14 days) *it seems that the amount of granulation tissue in all the groups decreases as compared with the 5-day groups, and the initially smaller granulation tissue amount in the control group attains the level of the hormone groups. The heaviest granulation*

*tissue is obtained by combined treatment, and thyrotropin seems to cause heavier granulation tissue than somatotropin.*

*Changes in the Tensile Strength of the Wound.* — For the 5-day test the force tearing the wound was measured by the different groups, according to the method described. The results are given in grams (gram force). For these results, too, the customary mean value and deviation calculations were made, and are presented in Table 3.

TABLE 3

BREAKING STRENGTHS OF SKIN WOUNDS

| Group | Number of Observations | Mean | Standard Deviation | Standard Error of the Mean |
|---|---|---|---|---|
| Control ..... | 11 | 238 | 97 | 29 |
| Somacton ..... | 10 | 305 | 87 | 28 |
| Actyron ..... | 10 | 266 | 135 | 43 |
| Somacton + Actyron . | 12 | 219 | 96 | 28 |

The statistical significance of the results was studied by variance analysis; the results for each of the three quantities are given in Table 4.

TABLE 4

ANALYSIS OF VARIANCE

| Source | Sum of Squares | Degrees of Freedom | Variance | Variance Ratio | P |
|---|---|---|---|---|---|
| Differences between groups | 44 896 | 3 | 14 965 | 1.36 | >0.1 |
| Of these: C contra S, A and SA ..... | 4 193 | 1 | 4 193 | (2.63) | >0.1 |
| S and A contra SA .... | 33 101 | 1 | 33 101 | 3.01 | >0.05 |
| S contra A ..... | 7 602 | 1 | 7 602 | (1.45) | >0.1 |
| Residual ..... | 429 387 | 39 | 11 010 | . | . |
| Total ..... | 474 283 | 42 | . | . | . |

The differences for which P is smaller than 0.05 can be considered statistically significant in the present material. The analysis shows no statistically significant differences in tensile strength. The

difference between the mean of the combined hormone group (IV) and the means of Groups II and III, however, is close to significance and suggests *that the tensile strength of the wounds of animals treated with somatotropin or with thyrotropin is greater than when the the hormones are given simultaneously.* The results are also given in Fig. 4 in order to enable comparison with similar drawings on the analysis of the various connective tissue components further below.

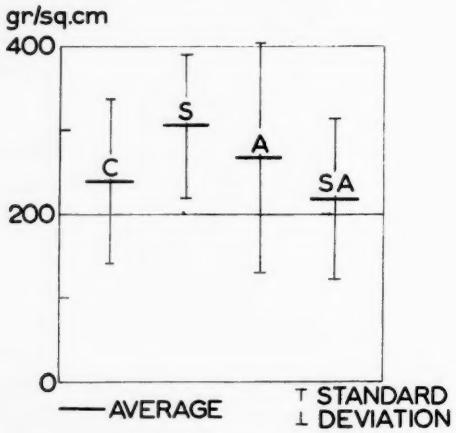



*Fig. 4.* — Tensile strength of the wound.
C: control group, S: somacton group, A: actyron group, SA: somacton + actyron group.

*Histologic Changes in Granulation Tissue: Quantitative Changes.* — The changes in the relative volume of the different granulation tissue components in the various groups, determined by the line sampling method described, are given in Table 5. This table compiles the computed group means for the control group and the different hormone groups, and the associated deviation calculations. To facilitate comparisons, the standard error of the difference between two means is ready computed. If the ratio between the difference of two means and its standard error in the present material is *2.12* or more the difference can be considered statistically significant.[1]

[1] For instance, for the homogeneous intermediate substance in the 5-day test, the difference of the means of Groups C and SA is 46—45; ratio 19/3.5 = 5.43, exceeds 2.12, and the difference is significant, $P < 0.05$ (t-test).

TABLE 5

PER CENT DISTRIBUTION OF THE TISSUE

| Group | Mean | Pooled Dispersion Statistics | | |
|---|---|---|---|---|
| | | Standard Deviation | Standard Error of the Mean | Standard Error of the Difference of the Two Means |
| *Ground substance, 5 days* | | | | |
| C = control | 45 | | | |
| S = somacton | 47 | 5.6 | 2.5 | 3.5 |
| A = actyron | 47 | | | |
| SA | 64 | | | |
| *Ground substance, 14 days* | | | | |
| C | 33 | | | |
| S | 19 | 5.4 | 2.4 | 3.4 |
| A | 49 | | | |
| SA | 59 | | | |
| *Fibrocytes, 5 days* | | | | |
| C | 14 | | | |
| S | 14 | 4.4 | 2.0 | 2.8 |
| A | 12 | | | |
| SA | 8 | | | |
| *Fibrocytes, 14 days* | | | | |
| C | 10 | | | |
| S | 10 | 2.0 | 0.9 | 1.3 |
| A | 7 | | | |
| SA | 7 | | | |
| *Giant cells, 5 days* | | | | |
| C | 5 | | | |
| S | 4 | 4.6 | 2.1 | 3.0 |
| A | 8 | | | |
| SA | 6 | | | |
| *Giant cells, 14 days* | | | | |
| C | 3 | | | |
| S | 5 | 2.4 | 1.1 | 1.6 |
| A | — | | | |
| SA | 3 | | | |
| *Inflammatory cells, 5 days* | | | | |
| C | 10 | | | |
| S | 12 | 2.5 | 1.1 | 1.6 |
| A | 11 | | | |
| SA | 4 | | | |

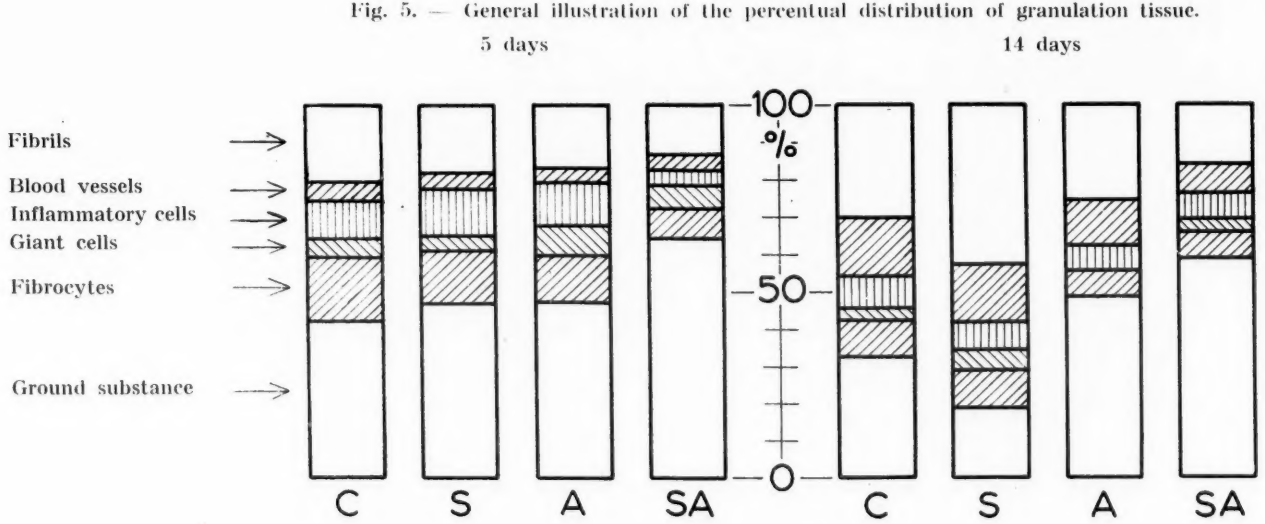
(Cont.)

| Group | Mean | Pooled Dispersion Statistics | | |
|---|---|---|---|---|
| | | Standard Deviation | Standard Error of the Mean | Standard Error of the Difference of the Two Means |
| *Inflammatory cells, 14 days* | | | | |
| C | 9 | | | |
| S | 7 | | | |
| A | 7 | 3.1 | 1.4 | 2.0 |
| SA | 7 | | | |
| *Blood vessels, 5 days* | | | | |
| C | 5 | | | |
| S | 4 | 4.8 | 2.2 | 3.1 |
| A | 4 | | | |
| SA | 4 | | | |
| SA | 4 | | | |
| *Blood vessels, 14 days* | | | | |
| C | 16 | | | |
| S | 16 | 4.3 | 1.9 | 2.7 |
| A | 12 | | | |
| SA | 8 | | | |
| *Fibrils, 5 days* | | | | |
| C | 20 | | | |
| S | 18 | 3.7 | 1.7 | 2.4 |
| A | 17 | | | |
| SA | 13 | | | |
| *Fibrils, 14 days* | | | | |
| C | 30 | | | |
| S | 42 | 3.1 | 1.4 | 2.0 |
| A | 25 | | | |
| SA | 16 | | | |
| SA | 16 | | | |

Fig. 5 shows the general picture of the percentual distribution of the listed 6 components of granulation tissue.

The *homogeneous intermediate substance*, most abundant in the 5-day test in each group, was found on special staining (described below) to consist mainly of the ground substance of connective tissue, and partly of empty tissue stomata from which the substance

Fig. 5. — General illustration of the percentual distribution of granulation tissue.




C: control group, S: somacton group, A: actyron group, SA: somacton + actyron group.

had disappeared or shrunk on fixation. In the 5-day test the control group showed the least amount of this substance, but only compared with the SA group is the difference significant.

In the 14-day test the relationship changed, the somatotropin group showing least and the combined group most of ground substance. Compared with one another, the differences between the groups are statistically significant.

*Connective tissue cells.* In the 5-day test the control group and the somatotropin group showed equal amounts of these cells and more than the rest of the *hormone groups, but only compared with the combined group* is the difference significant.

In the 14-day test the amount of connective tissue cells was again equal in the control and somatotropin groups and significantly higher than in the thyrotropin and combined group.

*Giant cells.* The variations in the amount of giant cells were not significant in either the 5-day or the 14-day test, although it seemed that all the groups had a greater number of giant cells in the 5-day than in the 14-day test.

*Inflammatory cells.* In the 5-day test these cells were significantly fewer in the combined hormone group than in the rest of the groups. In the 14-day test the differences levelled out.

*Blood vessels.* No significant differences in the number of blood vessels were observable in the 5-day test.

In the 14-day test the number of blood vessels increased in all the groups, but remained significantly lower in the combined group than in the control and somatotropin groups.

*Fibrils.* Their number seemed to decrease in the 5-day test compared with the control group, in the following order: somatotropin, thyrotropin and combined group. However, the difference is statistically significant only between the control and somatotropin groups as compared with the combined hormones group.

In the 14-day test the differences widened and changed. The somatotropin group showed the greatest number of fibrils, the other groups less, the order being: control group, thyrotropin group and combined group. The differences are significant in all the groups.

On the basis of this experiment, the hormones used seem to have the following effect on the quantitative changes in the various granulation tissue components:

*To start with, somatotropin does not seem to change the proportions between the components in the 5-day test, compared with the control group. Later, however, in the course of 14 days it increases significantly the fibrils and reduces the ground substance as compared with the controls. In other words, it seems to promote the maturing og granulation tissue.*

*With thyrotropin the differences as compared with the controls are not great initially. But it seems to induce a tendency towards reduction of collagen fibrils and connective tissue cells and towards an increase in inflammatory cells and ground substance. In 14 days this effect emerges distinctly, except that differences no longer occur in the amount of inflammatory cells. Hence thyrotropin seems to inhibit the maturing of granulation tissue and is antagonistic to the effect of somatotropin.*

*Administered together, the two hormones do not neutralize one another in effect, and it seems that somatotropin enhances the effect of thyrotropin both in the 5-day and 14-day tests. The quantity of connective tissue ground substance is greater still, and the amount of fibrocytes, collagen fibrils and blood vessels smaller still than in the group treated with thyrotropin alone.*

*Histologic Changes in Granulation Tissue: Changes Observable by Different Staining Methods.* — For the study of the different granulation tissue components the specially stained microscopic preparations were also examined.

*Pentachrome II Staining*, by which the various elements of the connective tissue can be shown up in a single section, revealed the following:

The 5-day test shows no significant differences between the control group and the somatotropin group.

In the group treated with thyrotropin, the poverty in fibrils emerges clearly. The amount of collagen fibrils is distinctly less than in Groups I and II. The homogeneous, structureless substance stains blue, showing up mainly as connective tissue ground substance.

In the combined group, in which the animals had been treated with the two hormones simultaneously, the occurrence of collagen fibrils is scantier than in the former groups. Elastic fibrils are hardly seen in the region of the granuloma. The structureless substance is blue in colour and hence obviously ground substance. The gauze fibres stained red, as they did in the previous groups. No orange-

Fig. 6. Cotton plug granuloma from the control rat. The gauze fibres have caused giant cell reaction and granulation tissue, with some ground substance of connective tissue and inflammatory cells. Pentachrome staining × 230.

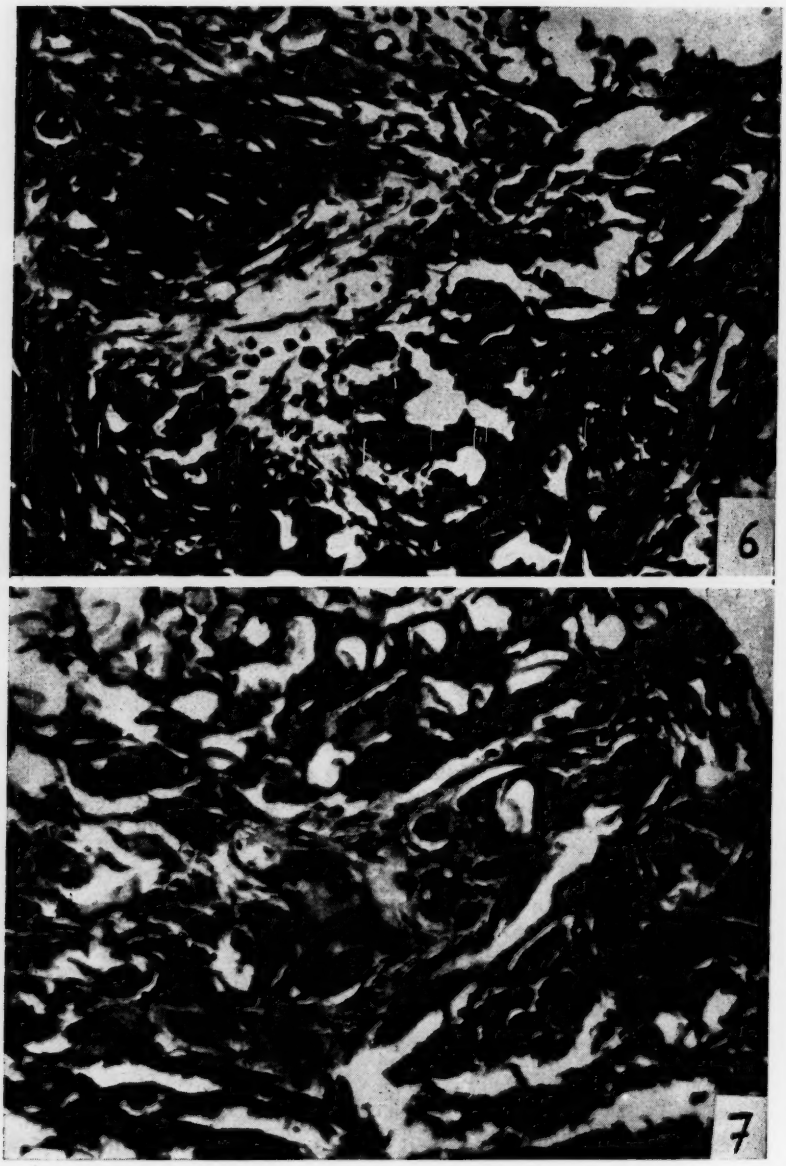
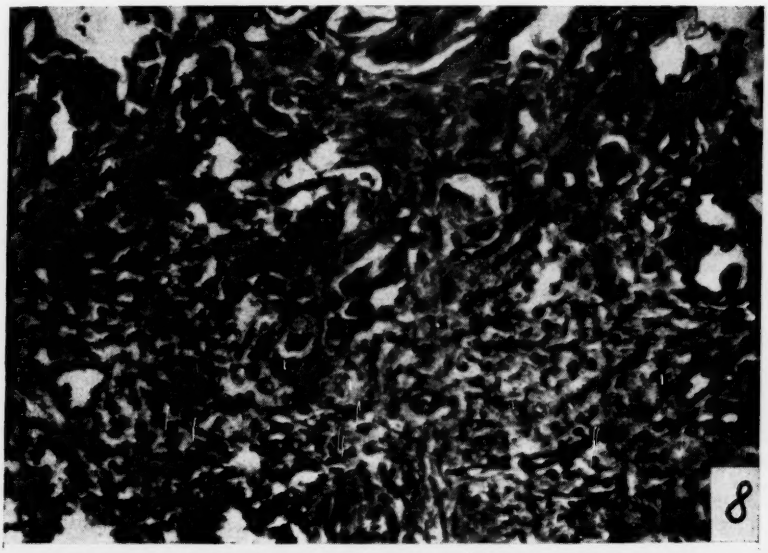



Fig. 7. — The effect of STH on granulation tissue. Note the increased amount of connective tissue fibrils and decrease in ground substance and inflammatory cells. Penta chrome staining, × 230.

Fig. 8. — The effect of TSH on granulation tissue. Note the increase in ground substance and decrease in blood vessels and collagenic fibres. Pentachrome staining, × 230.



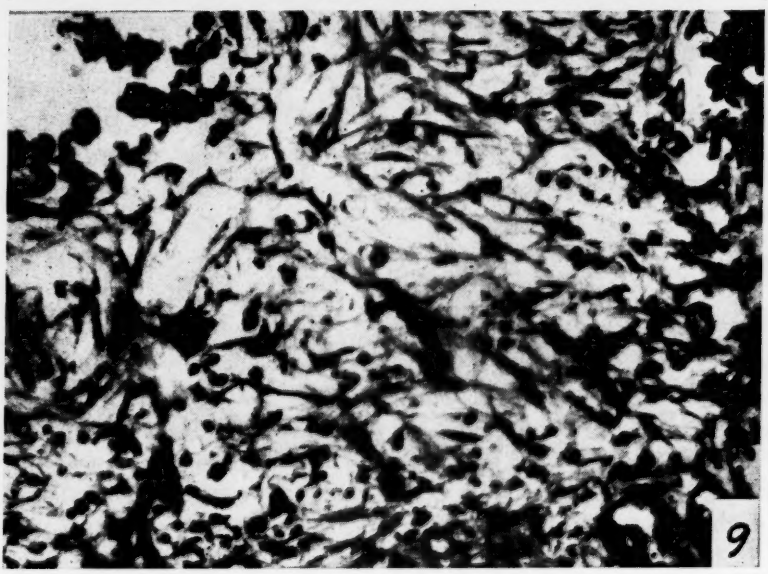




Fig. 9. — The combined effect of STH and TSH. Note the great increase in ground substance, the weak giant cell reaction and decrease in blood vessels and connective fibres. Granulation tissue is quite immature. Pentachrome staining, × 230.

yellow fibrinoid substances were present. The protoplasm of giant cells stained bluish or blue-green, like the ground substance.

In the 14-day test tissue containing collagen fibrils is noted around the granuloma, slightly capsule-like. Between the gauze fibres is a moderate amount of red collagen fibrils; they were practically absent in the 5-day groups. On the other hand, the blue colour indicative of ground substance between the gauze fibres has decreased considerably. Giant cells seem to be fewer; they have obviously already done their job.

In the group treated with somatotropin, the amount of red collagen fibrils is greater than in the previous group, and they tend to grow from the periphery of the gauze fibres towards the centre. The gauze fibres are split up into pieces smaller than before and seem usurated.

In the group treated with thyrotropic hormone nothing essentially new emerges on pentachrome staining. The amount of fibrils is scanty and that of ground substance great, in concurrence with the quantitative test.

The group treated with the two hormones shows, as in the quantitative study, that the number of fibrils is smaller than in the former groups.

*PAS Staining* for acid mucopolysaccharides showed that the protoplasms of giant cells and the homogeneous, structureless substance stain PAS-positive, corresponding to the components staining blue on pentachrome staining.

## DISCUSSION

A comparison of the results obtained by the different methods makes it clear that somatotropin increases the amount of granulation tissue initially, although the difference from the controls later disappears. At first the quality of the resulting granuloma does not differ from that present in the control group, but later it is richer in fibrils and contains less connective tissue ground substance than the controls. Hence somatotropin accelerates the growth of granulation tissue and matures it. This phenomenon is paralleled by the greater tensile strength of the wounds. Thus the present results are on the same lines as those reported by Taubenhaus & Amromin

(1950) and by Majarakis (1946), and supplement their results with tissue analyses.

With thyrotropin, the granulomas are at first larger than and later of the same weight as those of the controls. Although after 5 days the differences from the controls are not great there is a tendency towards a decrease in collagen fibrils and cells of the connective tissue and towards an increase in ground substance. In the group treated for a longer period the effect is similar but still more marked except that differences no longer occur in the amount of inflammatory cells.

Hence it is obvious that thyrotropin has an inhibitory effect on the maturing of granulation tissue. The ground substance of the connective tissue, primarily the mucopolysaccharides, increases at the expense of collagen fibrils and fibrocytes. The tensile strength of the wound is of the order found among the controls, but lower than that in the somatotropin group.

The results corroborate earlier observations that thyrotropin increases the mucopolysaccharides of the connective tissue. The discovery of its effect on the amount of fibrils and fibrocytes in the granulation tissue is new. The results also show that somatotropin and thyrotropin are antagonistic in their effect on granulation tissue.

This makes it somewhat paradoxical to find that combined treatment with thyrotropin and somatotropin by no means results in neutralization of the effect of both these hormones. Indeed, somatotropin rather enhances the effect of thyrotropin in both the 5-day and 14-day tests. The amount of connective tissue ground substance is greater still than in the thyrotropin group. On the other hand, the amount of fibrocytes, collagen fibrils in the connective tissue and blood vessels is small. The tensile strength of the wounds, although on a level with that of the control group, is lower than in the rest of the hormone groups.

The finding that somatotropin adds to the potency of thyrotropin seems, however, to be corroborated in another way also. In the caseinate amyloidosis studies by Peräsalo, Uotila & Virkkula (1956), not published yet, somatotropin enhanced the amyloidosis-inducing effect of thyrotropin. On the other hand, this effect seems to be dependent on the mutual quantitative proportions of the hormones concerned. Using a different dosage, Uotila, Peräsalo &

Vapaavuori (1955) found that somatotropin can inhibit the enhancing effect of thyrotropin on amyloidosis. On the other hand, the growth-promoting action of STH is enhanced by administering it in combination with thyroid-stimulating hormone (Marx, Simpson & Evans 1942).

A comparison of the above effects of somatotropic hormone and thyrotropin with the effect of cortisone on the connective tissue level shows interesting correlations. Cortisone inhibits the formation of both ground substance and connective tissue, and fibrocyte proliferation, and hence also the healing of the wound. Somatotropic hormone and thyrotropin obviously represent factors which do not cause inhibition of the healing process, although an effect promoting or inhibiting the maturing process can be produced on the quality of granulation tissue.

On the basis of the above, I consider it possible that thyrotropin and somatotropic hormone will gradually gain increasing importance in practical medicine and not least in surgery.

## SUMMARY

In the experimental conditions employed, somatotropin first increased the cotton plug granulation although the difference later disappeared. To start with, the granulation tissue was similar to that found in the controls; later it became richer in fibrils and contained less ground substance than that of the controls. There was a correspondence between this maturing effect of somatotropin and the increased tensile strength of the wound.

With thyrotropin the cotton plug granulomas were first heavier than those found in the controls; later the difference disappeared. In acute experiments thyrotropin had no appreciable effect on the quality of the granuloma; later, the fibrils and fibrocytes decreased and the inflammatory cells and ground substance increased. The tensile strength of the wound was lower than in the somatotropin group. Hence thyrotropin inhibited the maturing of granulation tissue.

The effects of somatotropin and thyrotropin on granulation tissue were antagonistic.

Somatotropin enhanced the inhibitory effect of thyrotropin on the maturing of granulation tissue; the amount of ground substance became greater still and that of fibrocytes, fibrils and blood vessels smaller still than in the group treated with thyrotropin.

Somatotropin and thyrotropin did not prevent the wounds from healing, as does cortisone; in the author's opinion, the quality of the granulation tissue can be affected by them to some extent — they can either promote or inhibit the maturing process;

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FROM THE MUNICIPAL BACTERIOLOGICAL LABORATORY, HELSINKI

# A STUDY OF THE OCCURRENCE OF POLIOMYELITIS AND OF NEUTRALIZING ANTIBODIES AGAINST THE THREE TYPES OF POLIOMYELITIS VIRUS IN FINLAND

BY

*KAISA LAPINLEIMU*

HELSINKI 1956

HELSINKI 1957
MERCATORIN KIRJAPAINO

# PREFACE

The present study was carried out in 1953—55 at the Municipal Bacteriological Laboratory, Helsinki.

I wish to extend my warmest thanks to the chief of the laboratory, Dr. N. Oker-Blom, M.D., for giving me the opportunity to carry out this scientific study, for his guidance and encouragement in the course of the work and for criticizing the manuscript.

In addition, I wish to express my sincere thanks to Dr. K. Penttinen, M.D., for his invaluable and encouraging help at the different stages of my work and for criticizing the manuscript.

My thanks are also due to Professor G. Olin, M.D., Professor S. Gard, M.D., and Dr. T. Wesslén, M.D., Stockholm, for their kindness in allowing me to study the tissue culture method used in their laboratories.

During the performance of my work the following persons have given me valuable help:

Professor V. Rantasalo, M.D., former chief of the Aurora Hospital and Dr. H. Nevanlinna, M.D., chief of the Red Cross Blood Service who placed at my disposal the patient material from Helsinki;

Dr. R. Pätiälä, M.D. who organized the collection of blood samples in the rural districts and kindly allowed me the use of reports to the State Medical Board;

the public health assistants, particularly Miss Astrid Holm, who collected the blood samples in the rural districts;

the chiefs and nurses from the maternity hospitals in Helsinki particularly Professor A. Turunen, M.D., and Professor P. Vara, M.D., from the Women's Clinic who helped me in obtaining embryos for the tissue cultures;

Mrs V. Perttunen and Miss S. Ahvenniemi who gave skilful assistance in the practical work;

Mr J. Kihlberg, M.A. who advised on the statistical treatment of the material;

Mrs E. Palmgren, M.A., and Mrs J. M. Perttunen who translated the survey of the literature and

Mr U. Attila, M.A. and Mr W. H. Dullforce, M.A. (Canterb.) who translated the rest of this paper into English.

I wish to express my thanks and gratitude to my colleagues and to all those who have in various ways assisted me.

Financial support for this work was granted to me by the Sigfrid Juselius-Foundation, the Concordia-Union, the University of Helsinki and the Society of Young Physicians (NLY), for which I am grateful.

Helsinki, Oktober 1956.

*Kaisa Lapinleimu*

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# INTRODUCTION

In statistical reports on poliomyelitis attention has been called to the low incidence of poliomyelitis in Finland as compared with the Scandinavian countries.

The reasons for this are unexplained, although it has been suggested that the difference might be ascribable to differences in the occurrence of poliomyelitis neutralizing antibodies. Formerly the determination of poliomyelitis neutralizing antibodies required the use of a great number of monkeys as experimental animals, which made it impossible, for practical reasons, to carry out antibody determinations in Finland.

This difficulty has recently been overcome through the introduction of the tissue culture technique into the study of the poliomyelitis virus. In 1949—50 Enders, Weller and Robbins developed this method for cultivating poliomyelitis viruses in vitro. The observation that these agents destroyed the cells in which their multiplication occurred made it possible to employ tissue culture methods to demonstrate the presence of poliomyelitis neutralizing antibodies.

The object of the work now reported was to study the incidence of poliomyelitis neutralizing antibodies in certain localities in Finland in different age groups, and to compare these findings with the incidence of poliomyelitis.

# SURVEY OF THE LITERATURE[1]

## EPIDEMICS OF POLIOMYELITIS

Poliomyelitis probably occurred as a sporadic disease even before Christ, but epidemics of poliomyelitis attracted the attention of physicians for the first time in the nineteenth century. The first important epidemics occurred in Scandinavia. In 1905 a severe outbreak occurring in Sweden was successfully analysed by Wickman (178). Although the cause of the disease was unknown, he emphasized in particular its infectious nature, its transmission from one person to another, and the significant role of abortive cases in its spread.

Some 50—60 years ago, poliomyelitis assumed an epidemic, instead of an endemic, pattern both in Scandinavia and the United States (45, 121). During subsequent years, poliomyelitis epidemics occurred in many European countries as well as in Canada, Australia and New Zealand (40). These outbreaks attracted considerable attention and gave rise to the widely accepted belief that poliomyelitis is principally a disease of the temperate zones, being rare in the tropics. Later investigations have shown, however, that the poliomyelitis virus is common in tropical areas, though it is more or less hidden (119), and though the disease is endemic there and thus does not attract the same degree of attention as when it assumes an epidemic pattern, and that every year the virus is responsible in various areas for sporadic cases in infants, in particular (120). This has been shown to be the case in Cuba (122), Egypt(123), Japan (118), the Philippines (141), and in certain native communities in Asia and Africa (154).

[1] This survey was completed in December, 1955.

The clinical disease comes to the surface when susceptible immigrants, such as foreign soldiers, enter such an endemic zone. During World War II it was observed in Egypt (120, 123), in the Philippines (141), and in China and Korea (141) that morbidity rates were extremely low in the native population, infants alone being involved (154), whilst among the foreign troops the rate of the disease was as much as ten times higher than the level in the corresponding age group in their own country (141).

Paul (119) suggested that the endemic pattern of poliomyelitis occurring today in these »primitive» communities corresponds to the state prevalent in the nineteenth century in those countries where epidemics now occur. Gear (45) assumed that in the future epidemics will become a worldwide phenomenon.

In Sweden, it has been observed that epidemics occur at intervals of 5—8 years (112).

In areas from which reliable reports on poliomyelitis epidemics are available a steady increase in paralytic rates has been observed, in particular during the last 20—30 years (40, 45, 121).

Another important change that has occurred in the pattern of the disease during the last few decades is a shift towards the older age groups. During the first epidemics, poliomyelitis almost exclusively attacked children under 5 years old (81, 178). This is still the case in the native communities in Africa and Asia, where about 90 per cent of paralytic cases occur in the age group under 5 (45, 118, 119, 154). According to morbidity reports from Europe and the United States, the proportion of children under 5 years old among the paralytic cases has decreased so much that the highest paralytic rate occurs in the age group 5—9 years (50, 131, 174). In many areas of the United States, 70—80 per cent of the paralysis occurs in patients over 5 years old, and 25—35 per cent in patients over 15 (133), in Sweden even 50 per cent in patients over 15 (113). The proportion of adult paralytic cases has increased, particularly in those areas where epidemics occur (40).

The cause of this change in the character of clinically diagnosed poliomyelitis has not been identified, but various hypotheses have been advanced. It has been suggested

1) that mutations of the poliomyelitis virus have resulted in strains causing paralytical disease more frequently than the previous strains (18, 142);

2) that improved criteria for diagnosis and more complete reporting are responsible for an apparent change (29, 123);

3) that an improved standard of living, improved hygiene, and better public health care are responsible (45, 112, 121).

The last assumption would explain the modification of poliomyelitis from an endemic to an epidemic disease and the change in its age distribution. With improved hygiene, the chances of infants being exposed to contact with the virus are decreased, and this accounts for the reduced morbidity during the first years of life. When the virus spreads in modern civilized communities, the large number of susceptibles causes an epidemic, whereas in primitive conditions the disease gains no foothold owing to the lack of susceptible persons capable of transmitting the virus. The increased rates of paralytic poliomyelitis in adults are referable to the fact that the later in life infection with the virus occurs, the greater is the risk of paralysis (43, 98, 129).

This assumption is substantiated by the following observations:

1. Epidemic poliomyelitis occurs in countries with a high standard of living, whilst it is endemic in primitive communities (154).

2. In cities, where the population as a rule is crowded, poliomyelitis occurs principally in the youngest age groups, and the shift towards the older ages is less marked than in the rural districts (60, 112, 117, 131).

3. In the lower economic groups infection occurs at a lower age level than in the upper economic groups in the same community. Collins (24) found that paralytic rates were highest in the former from 0—4 years, in the latter from 10—14 years.

## THE EXPERIMENTAL INVESTIGATION OF POLIOMYELITIS

### *Experiments on Monkeys*

Although, at the end of last century, it was assumed on clinical grounds that poliomyelitis is an infectious disease, this assumption was not proved until Landsteiner and Popper in 1909 succeeded in producing a paralytic febrile disease in a monkey by injecting a spinal cord suspension from a patient who had died from poliomyelitis. In 1910 Flexner and Lewis, and in the subsequent years

many other investigators, showed that this disease is caused by a filtrable virus.

The agent responsible for poliomyelitis and an animal species susceptible to infection having been detected, experimental research commenced. Although it was restricted by the expense and difficulty associated with procuring and maintaining the animals, the physical and chemical properties of the poliomyelitis virus were evaluated during this »monkey era». Furthermore, the clinical picture was studied in these experimental animals, data being collected on the incubation time and on the paralytic and non-paralytic phases of the disease. Virus was detected in the faeces of the infected monkeys, in the mucous membrane of the throat and intestine, in the lymphatic glands, in the nerves, in the bone marrow and brain, in secretions from the throat and nose, and finally also in the blood. Furthermore, neutralizing antibodies to the poliomyelitis virus were detected in the blood and in secretions from the nose and throat of monkeys convalescent after poliomyelitis.

In various parts of the world different strains of the poliomyelitis virus were isolated in monkeys. With regard to infectivity (1, 167), virus activity (72, 128), and antigenicity (20, 72, 86, 168) they differed from each other. Therefore, in 1948 the National Foundation for Infantile Paralysis appointed a Committee on Typing, the task of which was to classify the poliomyelitis viruses into subgroups on the basis of their immunological properties, in particular. The immunologically distinct strains called Brunhilde, Lansing and Leon were chosen as prototypes. Employing cross-immunization and -neutralization, four different university teams carried out research for three years (25). To begin with, 100 different strains of virus, isolated at various times in various parts of the world, were studied. Immunologically 85 of these were identified as Brunhilde, 12 as Lansing, and 3 as Leon. The first-mentioned strains were called type I, the Lansing-type strains were called type II, and the last-mentioned viruses were called type III. It appeared that type I was the agent most commonly responsible for clinical poliomyelitis, but since random selection had not been employed, this conclusion could not be regarded as established (150). In 1953 2200 strains of poliomyelitis virus were isolated, 84% belonging to type I, 7% to type II and 9% to type III (41).

### *Experiments on Rodents*

In 1939, Armstrong was able to infect cotton rats with the Lansing strain of poliomyelitis virus injected intracerebrally (1). Subsequently white mice (2), and hamsters (132), were successfully infected. Typical changes were also caused in these rodents with other strains of the Lansing type, but not with any strain of Brunhilde or Leon type.

The observation that mice could be substituted for monkeys considerably facilitated the experimental study of poliomyelitis. Since antibody tests presuppose an abundant supply of experimental animals, their performance on a large scale became possible when Armstrong (48) showed that identical results were obtained in mice and monkeys. The value of the epidemiological antibody investigations using mice was limited, however, by the fact that only the occurrence of antibodies to the strains of the Lansing type could be elucidated, and the role of the latter in the general etiology of poliomyelitis was not understood (126).

It has subsequently also proved possible to infect mice (88) and hamsters (134) with the Leon strain, and mice (89) with the type I Mahoney strain.

### *Experiments on Embryonated Hen's Eggs*

After many unsuccessful attempts, the type II MEF1 strain, which had first been carried through many passages in suckling hamsters, was successfully cultured on embryonated hen's eggs (21, 140).

### *Experiments with Tissue Cultures*

An entirely new stage in the experimental investigation of poliomyelitis began when Enders, Robbins and Weller in 1949 succeeded in growing viruses of type II in tissue cultures prepared from human embryonic tissues.

Numerous investigators had previously tried to cultivate poliomyelitis virus in tissue cultures. Of these experiments, those of Sabin and Olitsky (146) have been repeated. These workers were able to grow the virus in human embryonic brain tissue, but not

in extraneural tissues. Attempts to repeat the experiments of other investigators have been unsuccessful.

Having first grown the Lansing strain of the poliomyelitis virus in vitro in the skin-muscle tissue of human foetuses, Enders, Robbins and Weller showed in 1949 that it was possible to cultivate the Brunhilde type (172), and in 1951 the Leon type (173), under the same conditions. They also showed that these viruses grow in human postnatal tissues, *e.g.* the prepuce (172), the kidney (173), and the uterine myometrium (139). They observed that the host cells were destroyed by the multiplying virus, and that the cytopathogenic effect of the latter was inhibited in the presence of homotypic serum (137).

These observations were confirmed by numerous investigators using cultures of monkey tissues (31, 35, 84, 162, 182), or human tissues (39, 155, 162).

Tissue culture was thus substituted for experimental animals, the cyto-pathogenic effect on the cultured cells corresponding to the paralysis caused in animal experiments. In respect of sensitivity, the tissue culture proved to be equal to the monkey and the mouse (83).

### *Methods of Tissue Culture*

Tissue culture methods based on four different principles have been used:

*Suspended-cell or Suspended-fragment Culture.* — Detailed instructions for the preparation of these cultures have been published in the following papers: 162, 173.

Various human embryonic tissues have been used, *e.g.* skin, muscle, internal organs, and brain (172, 173). Furthermore, postnatal human prepuce (172), human (155) and monkey (182) testis, and human (138) and monkey (180) kidney have been employed. The best cultures, yielding the highest virus titres, have been obtained from the kidney (39).

The growth of the virus is indirectly determined from its influence on the pH of the culture as compared with control cultures (39, 173).

This method is used in the production of large quantities of

poliomyelitis virus, *e.g.* in the manufacture of vaccines or other virus dilutions of high titre.

*Fixed-cell or Fixed-fragment Tube Culture.* — The cells or tissues are cultivated on clotting plasma, which causes them to adhere to the walls of the tube. One advantage of this method is that the cytopathogenic effect of the growing virus on the cells may be directly visualized when the test tube is placed under the microscope. These cultures are grown either in roller tubes or in stationary tubes.

The *roller-tube* technique was first used by Gey and Bang (46) in the culture of other viruses. In 1950 Robbins, Enders and Weller (137) adapted it to the propagation of poliomyelitis viruses. For details the reader is referred to their paper (139). In addition to the tissues employed in the suspended-cell cultures, human tonsillar (39) and myometrial tissue (139) have been successfully used. With the roller-tube system, results are more rapidly obtained and more readily recognized than in the suspended-cell culture (139). Of the tissues employed, the kidney has proved to be the most satisfactory in these cultures, too (33).

In the *stationary* fixed-cell tube culture the same tissues are used, and the results obtained by the two techniques are largely equivalent, although the yield of virus seems to be somewhat higher when the cultures are rolled (100). The stationary method is technically easier. Furthermore, it has been simplified by preheating the tubes to 45°C. (103) and then immediately introducing renal tissue, the epithelial cells of which adhere to the walls of the tube without the addition of plasma.

*Trypsinized-cell Culture.* — Dulbecco and Vogt (30) developed a method by which it is possible accurately to determine the number of infective particles present in a virus suspension. Renal tissue is treated with trypsin, and a monolayer of cells is established in a Petri dish. A weak virus dilution is added, and the culture is covered with a layer of a nutrient agar mixture. On account of the cell destruction due to the multiplication of the virus, plaques are formed. Dulbecco showed that each plaque was caused by a single infective particle.

By this method it is possible to observe virus mutations, to determine viral infectivity, and to measure the concentration of antibody.

Youngner (180) has further elaborated this technique by preparing tissue cultures containing a given number of trypsinized monkey kidney cells in one layer. For details regarding the preparation of these cultures, the reader is referred to the original paper. Youngner's method appears to be superior to the previous techniques. The cell cultures are readily prepared, and the method is economical, 800—1000 cultures being obtained from one monkey.

*Cell Cultures Continuously Maintained in Laboratories.* — Gey (47) have maintained the so-called HeLa cells from a cervix carcinoma in continuous cultivation since February, 1951. Detailed instructions on the cultivation of HeLa cells and the adaptation of these cultures to the study of poliomyelitis have been published by Scherer, Syverton and Gey (153), and Syverton, Scherer and Elwood (163).

The use of HeLa cells has many advantages. They are readily obtainable from laboratories. Being epidermal cells they adhere to glass surfaces without any plasma. Furthermore, results are rapidly obtained owing to the sensitivity of the HeLa cells to the poliomyelitis viruses. Hence the use of these cells has been widely adopted in research on poliomyelitis.

Other malignant cells (159) and normal human embryonic cells (23, 79, 160) have also been cultivated continuously, but in the study of poliomyelitis they have not proved superior to HeLa cells or Youngner's renal tissue method.

*As culture media,* various buffered saline solutions have been used. The majority of workers have employed Hanks' solution with the addition of ultra-filtrate of bovine serum, horse serum and bovine embryonic extract (139), or ultra-filtrate of bovine serum together with enzymatic hydrolysate of lactalbumin (100). Parker prepared a widely used mixture of various vitamins and hormones, known as mixture 199 (106). In routine work, Enders also used bovine amniotic fluid together with horse serum and bovine embryonic extract (32).

In the investigation of poliomyelitis, tissue cultures have been used for the following purposes: isolation of the virus from the faeces or from infected tissues and secretions, titration of the virulence of the virus, systematic study of the factors involved in its multiplication, and observation of the effect of various drugs on the virus. Furthermore, tissue cultures have been employed in the

production of large quantities of virus dilutions of high titre, as in the production of vaccine and in the preparation of antigen for complement fixation tests. Since it was found that homotypic antibody inhibited the effect of poliomyelitis virus in tissue cultures, the latter have also been adapted to virus typing, to the assay of neutralizing antibodies, and to titration of the increase in antibodies developing after infection or vaccination.

## IMMUNITY

Soon after the detection of the poliomyelitis virus, it was observed that monkeys which had recovered from paralytic poliomyelitis were subsequently resistant to infection (38, 85). Simultaneously it was found that the sera of these resistant monkeys contained neutralizing antibodies to the poliomyelitis virus. According to some investigators, these were present by the 6th day after infection (15), whilst according to others they developed at various times, but never until 2—3 months after infection (57, 147). By contrast, in human beings who had recovered from a paralytic attack, neutralizing antibodies were demonstrable after 3 (75) to 14 (59) days.

During the subsequent decades, conflicting results were, however, also reported. Some workers were not able to demonstrate safe resistance in monkeys which had suffered from paralytic poliomyelitis (37, 73, 128). The recurrence of paralytic attacks was also observed in man. Fischer and Stillermann surveyed 26 cases from the literature and reported 4 cases of their own (36). According to other writers, neutralizing antibodies do not occur after paralytic poliomyelitis, or are present in equal amounts in the acute and convalescent stages (17, 170). Others observed antibodies even in the initial stage of the disease in 50 per cent of paralytic cases in man (52). Some writers alleged that after a paralytic attack resistance was demonstrable in monkeys prior to the development of neutralizing antibodies (147).

Furthermore, the sera of subjects who had not suffered paralytic poliomyelitis was found to contain antibodies in the same amounts as the sera of those who had suffered an attack of this disease (52, 116), and it was found that the percentage of sera with neutralizing antibodies increased with age (171). Circulating polio-

myelitis neutralizing antibodies were also encountered in many native populations (69, 87, 115) in whom poliomyelitis did not occur clinically. Hence it is hardly surprising that the conclusion was drawn that immunity to poliomyelitis is not to any appreciable degree dependent on the presence of neutralizing antibodies.

Simultaneously, however, other investigators postulated that there is an obvious correlation between infection and the occurrence of neutralizing antibodies (4, 6, 58, 74, 78, 166).

Owing to these conflicting results, opinions differed with regard to the development of neutralizing antibodies. Jungeblut assumed that their development is associated with physiological maturity (71). Other writers suggested that it is due to nonspecific infection, some other chemically related antigen (3) inducing the formation of antibody, or at least a change in the serum globulin (19) identical with that caused by the poliomyelitis virus. Other investigators were adherents of the theory that specific infection is the causative factor, the occurrence of neutralizing antibody being due to previous contact with the poliomyelitis virus.

Nearly 40 years elapsed before these contradictions were clarified and the specific infection theory became universally accepted. According to this theory the development of neutralizing antibodies is a sign of previous infection with poliomyelitis and follows the same pattern as in other viral diseases. The long delay in the disentangling of this problem was largely due to the fact that experiments had to be performed on such expensive animals as monkeys which, moreover, were difficult to procure, and the conflicting results are referable to the fact that the different types of poliomyelitis virus had not been identified, and that an accurate, quantitative method of determination of neutralizing antibodies was lacking. Antibody tests were performed with standard virus strains, which are now identified as type II. Quantitative investigations were carried out using undiluted serum and varying amounts of virus. Subsequently it has been shown in parallel experiments that it is not possible by this method to demonstrate any difference between acute and convalescent phase sera; this can only be done by neutralization tests using serum dilutions and a given amount of virus (158).

## *Neutralizing Antibodies in the Serum*

### *The Occurrence of Neutralizing Antibodies in Clinical Poliomyelitis*

The first investigations on neutralizing antibodies that were undoubtedly performed with the right type of virus were published in 1948 by Hammon and Roberts (54), who studied the neutralizing antibodies present in patients' sera using virus isolated from their faeces. They showed that antibodies were present immediately after the onset of a paralytic attack and increased in amount during subsequent weeks. These results were confirmed by others, and it was found, moreover, that in the same patients the development of neutralizing antibodies could not be demonstrated when laboratory strains (type II) of the poliomyelitis virus were used in the tests (158).

The development of neutralizing antibodies in clinical poliomyelitis has been quantitatively studied by other workers, too (10, 97, 99, 102, 176).

In 1954, it was established that in manifest cases of paralytic poliomyelitis an increased amount of neutralizing antibodies is not invariably demonstrable in convalescent phase serum as compared with acute phase serum, even when the tests have been properly performed. It was suggested that in these cases the rise in antibodies had occurred prior to the appearance of manifest signs (102).

These results were explained by experimental investigations on monkeys. When the animals had been infected with poliomyelitis virus, neutralizing antibodies developed during the second week of incubation (91, 92). High titres were noted immediately before, or immediately after, the paralytic stage (11, 91, 92).

When, in 1952, Horstmann (61) and Bodian (11) had demonstrated the presence of virus in the circulating blood of monkeys even by the 4th—7th day after ingestion of virus, the early development of neutralizing antibodies was theoretically explained. Simultaneously Horstmann and Bodian, studying the contacts of poliomyelitis patients, observed the occurrence of a similar viremia in man during the early stage of incubation (14, 63).

### *The Occurrence of Neutralizing Antibodies in Subclinical Poliomyelitis*

Subclinical infection has been experimentally induced *in monkeys*, in particular by oral administration of virus. During the second week, virus was found in the faeces (61). Viremia has been observed on the 4th—6th day (11, 61). Following the viremia, neutralizing antibodies were demonstrated in the serum just as in clinical poliomyelitis, although no clinical symptoms of the disease were observable (11, 12, 64, 68, 92, 96, 166).

*In man*, subclinical poliomyelitis infection has been studied by several investigators (14, 16, 76). A total of 272 children without Lansing antibodies were observed over a period of 2 years, using the neutralization test on mice. During this time 51 children developed neutralizing antibodies without showing any symptoms of the disease (169).

When the use of tissue cultures had been adopted, Melnick and Ledinko (98) similarly investigated all three neutralizing antibodies to the poliomyelitis virus. They were able to show that during an epidemic caused by type I, children who were free from clinical disease developed neutralizing antibodies to both type I and type II. This was also shown by Oker-Blom during the Aaland epidemic in 1953 (110).

In studies on the contacts of poliomyelitis patients, the virus was isolated from throat swabs (63, 177) and from the faeces (16, 63, 177) and blood of children (14, 63) without any clinical symptoms. Simultaneously a rise in neutralizing antibodies to the same type was observed (14, 16, 176).

In the lower economic groups, where the risk of infection and thus of subclinical disease are greater owing to the comparatively poorer hygienic conditions, the percentage of children with neutralizing antibodies is higher than in the upper economic groups in the same community (56, 80, 98).

### *The Occurrence of Heterotypic Neutralizing Antibodies in Poliomyelitis*

In 1949, when he was typing virus strains in *monkeys*, Bodian (9) found that a recurrent attack of paralysis did not result when a homotypic strain was used a second time, whilst infection with a

heterotypic strain brought about the recurrence of paralysis in 50 per cent of cases, and paralysis in 90 per cent of the controls. Some kind of protection had thus been derived from the heterotypic virus first employed. Howe (66), who studied the development of neutralizing antibodies in monkeys, also observed a rise in heterotypic antibodies, too, inasmuch as the antibody to type I increased in response to infection with type II, and the antibody to type II increased in response to infection with type III.

The development of heterotypic antibodies has likewise been observed in *man*. In investigations on the neutralizing antibodies of patients suffering from paralytic poliomyelitis, Steigman and Sabin (158) observed homotypic antibodies to type I in the serum even in the initial stage of the disease, and within 1—3 months 6 patients out of 24 had also developed heterotypic antibodies to type II. Sabin (143) observed that out of 18 patients with infection due to type I, 11 also developed neutralizing antibodies to type II within 2—4 weeks from the onset of the disease. The latter disappeared within 3 months, whilst the homotypic antibodies persisted. In explanation of these findings, it was assumed that types I and II, or at least the strains in question, have antigens in common. An increase in heterotypic neutralizing antibody, although in lower titre than the homotypic antibody, was observed by Salk in vaccination experiments (151), and by several other investigators, who assumed the explanation to be that the different types have some common antigens (99, 102, 110, 175).

That an antigenic relationship exists between all types of poliomyelitis virus is clearly demonstrable by the complement fixation reaction (22, 161). Melnick's complement fixation tests (95) revealed that types I and II, and types II and III, have the same antigens. By contrast, types I and III do not appear to be equally closely related antigenically.

Melnick and Ledinko published a report (98) on a poliomyelitis epidemic caused by type I, during which children who were free from clinical symptoms of the disease also developed neutralizing antibodies to type II. The writers suggested that in these cases multiple, inapparent infection with both types of virus was present, although they had not been able to isolate type II from any patient.

*The Titre of Neutralizing Antibodies in Poliomyelitis*

The Committee on Typing of the National Foundation for Infantile Paralysis (25), appointed in 1948, recommended the use of diluted sera and a constant dosage of virus, containing 100 $TCD_{50}$, in titrations of antibodies using the neutralization test.

The first antibody titrations thus performed revealed that neutralizing antibodies were present in the blood as early as 12 hours after paralysis had occurred (158), and that they increased in amount during the subsequent weeks (54, 158). To begin with, it was believed that the neutralizing antibody titre was higher in the paralyzed cases than in the nonparalyzed, but subsequently it has been found that in subclinical cases titres are quite as high as in clinical cases both during epidemics (98) and when there is no epidemic (16, 169).

There are great antigenic differences between type-specific strains, too (13, 26).

A similar lack of correlation as appeared between the antibody titres of different clinical stages was revealed between the titres due to the different types (26, 99).

By contrast, an obvious difference was discernible between the amounts of homotypic and heterotypic antibodies. Investigating the development of neutralizing antibodies in subclinical cases during an epidemic caused by type I, Melnick and Ledinko (98), for instance, found that in a sample of children whose titre of neutralizing antibodies to type I was 1: 100—1: 1000 in 88 per cent of cases, the titre of antibodies to type II was 1: 100 in 49 per cent or less, 1: 100—1: 10 in 10 per cent, and 1: 10 or less in 41 per cent. Similar results showing that the titre of heterotypic neutralizing antibodies is lower than the titre of homotypic antibodies have been published by several other investigators (66, 102).

The variations observed in the titre of neutralizing antibodies are also dependent on whether the tests are performed with the infective strain, or with some standard, although homotypic, strain. Sabin and Fieldsteel (145) showed that monkeys who had been given a diet containing the Y-SK strain of virus belonging to type II, developed type II antibody yielding a high titre when tested with the Lansing strain, but a low titre in tests with the MEF1 strain performed under otherwise identical conditions.

### *Persistence of Neutralizing Antibodies in Poliomyelitis*

Melnick (94) was able to show that neutralizing antibodies persisted in *monkeys* for at least 6 years, although the titre dropped somewhat during this time. Persistence of neutralizing antibodies, and a drop in the level within 10 months—2 years, has been demonstrated by other investigators also (15, 66, 68, 105).

In *man*, neutralizing antibodies have also been proved to persist for years. Paul demonstrated persistence for 20 years (127), Turner *et al.* for 7 years (169), Melnick for 3—4 years (94), Winsser and Sabin for 3 years (179), Koprowski *et al.* for 3 years (77). Other workers have studied the occurrence of neutralizing antibodies over shorter periods (8, 67, 152, 176).

Howe (66) postulated that the neutralizing antibody titre gradually drops, and that the original level is restored by repeated infections acting as booster dosages. A drop in the antibody level within 8 months has also been observed in Salk's vaccination experiments (151).

This theory does not, however, account for the fact that in 50 per cent of the isolated Eskimo population of Alaska, Lansing antibodies have persisted for 20 years at as high a titre as 1: 100 apparently without the introduction of any booster dosages (127).

In 14 cases of poliomyelitis in children, Miller and Wenner (102) did not observe any drop in the homotypic neutralizing antibody titre during a period of 18 months.

### *Tissue Immunity*

It has been shown that monkeys paralyzed as a result of intracerebral or intranasal infection with poliomyelitis virus are resistant to later intracerebral or intranasal infection with the same strain, although neutralizing antibodies are not demonstrable in their sera (70, 147).

In the spinal cord of monkeys intracerebrally infected with Lansing virus, Morgan (104) found neutralizing antibodies in abundance, although none was demonstrable in the serum. In the spinal cord of monkeys similarly infected with other strains of the virus no neutralizing antibodies were encountered (148).

This resistance has been ascribed to tissue immunity which is not due to the presence of neutralizing antibodies in the blood stream (144).

## GEOGRAPHICAL DISTRIBUTION OF NEUTRALIZING ANTIBODIES TO POLIOMYELITIS

The occurrence of neutralizing antibodies to the poliomyelitis virus constitutes evidence of past infection either at a clinical or a subclinical level. Even if immunity, perhaps, comprises certain factors in addition to neutralizing antibodies, there is reason to recall the statement made by Paul: »There is a general pattern of relationship which allows one to use this approach in poliomyelitis epidemiological surveys when determining the »immune status» of a given population» (121).

It has been established that neutralizing antibodies to poliomyelitis are transmitted through the placenta and gradually disappear by the 4th—6th month of postnatal life, and that actively acquired neutralizing antibodies appear at different age levels in different communities (121), just like the neutralizing antibodies to other diseases.

For investigations on immunity, experimental animals are required in large numbers. Hence it is not surprising that during the »monkey era» of research on poliomyelitis such studies could only be performed on a limited scale (5, 55, 58). During the »mouse era» in the nineteen-forties, studies on immunity were only concerned with the Lansing type (7, 55, 56, 80, 116, 126, 142, 169, 171). Since in given communities a clear correlation was found to exist between the age groups with Lansing antibodies and the incidence of diagnosed cases, the conclusion was drawn that the occurrence of Lansing antibodies corresponds to the occurrence of neutralizing antibodies to the types of virus responsible for epidemics (51, 126, 169).

The adoption of the tissue culture technique made it possible to study the occurrence of neutralizing antibodies to all three types in various age groups in different communities (28, 42, 62, 98, 101, 110, 114, 124, 156, 164).

The investigations on the occurrence of neutralizing antibodies have shown that, apart from isolated areas, the poorer the hygienic

conditions, the earlier is the age incidence of neutralizing antibodies to poliomyelitis. It also appears that in the tropics where clinical poliomyelitis is rare, infection is widespread, and even hyperendemic (45). In countries where the hygienic conditions are no longer primitive, these antibodies develop in later age groups. In such a population the number of susceptibles is large, and thus the outbreak of an epidemic becomes possible. The results of these investigations lend support to the hypothesis that poliomyelitis epidemics are a result of improved hygienic and public health conditions.

## THE OBJECT OF THE PRESENT INVESTIGATION

The literature on poliomyelitis prompted an analysis of the situation in Finland. It seemed to be of interest to ascertain whether, *with regard to the occurrence of epidemics and the shift towards older age groups, the incidence of the disease has followed the same course in this country as in other civilized countries.*

It has been established that the presence of neutralizing antibodies to the poliomyelitis virus is a sign of previous infection, either clinical or subclinical, and that consequently in countries with a high standard of living and of hygiene, neutralizing antibodies develop later than among peoples living in primitive conditions.

Since no data were available in regard to neutralizing antibodies to poliomyelitis in Finland, attention was centred on their occurrence.

Thus the main questions to be answered were the following:

*In which age groups do neutralizing antibodies to poliomyelitis develop in this country?*

*Is there any difference between the urban and rural districts with regard to the age incidence of neutralizing antibodies?*

*Do the various rural communities differ in this respect?*

*What is the relationship between the incidence of manifest poliomyelitis and the occurrence of neutralizing antibodies to poliomyelitis?*

# PRESENT INVESTIGATION

## SOME EPIDEMIOLOGICAL PATTERNS OF POLIOMYELITIS IN FINLAND

### GENERAL INFORMATION

The land area of Finland is 305,396 km² and its population, according to registration lists at the beginning of 1954, was 4,203,700 (157). The population density, on an average 13.8 per km², amounts to 25—30 per km² in Southern Finland (73 per km² if the capital is included), to 15 per km² in Central Finland and to 1.9 per km² in Lapland.

The population is rather homogeneous, showing no great differences in regard to race or social status. About two thirds of the population live in rural surroundings, living mainly by agricultural and forest work.

The largest city is Helsinki with 400,000 inhabitants. Only two other cities have more than 100,000 inhabitants, the rest (32 cities) ranging below 50,000.

### THE MORBIDITY OF POLIOMYELITIS

According to Halminen (49), poliomyelitis epidemics have occurred in Finland since 1905.

From that date poliomyelitis cases have been reported to the State Medical Board, but before the autumn of 1953 no distinction was made in the reports between paralytic and nonparalytic cases. The following statistics on Finland have therefore been compiled from all the reported poliomyelitis cases. Only the data for the whole country in 1954, and for the city of Helsinki in the period 1944—54 indicate paralytic cases alone. The paralytic cases in

Helsinki could be separated by means of the case histories kept at the Aurora hospital (hospital for infectious diseases in Helsinki).

In calculating morbidity, only paralytic cases should be considered, since they provide the safest basis for a comparative study of the frequency of poliomyelitis infections. In Finland, quite a number of nonparalytic poliomyelitis cases remain unreported. In the rural regions a physician is not often consulted for cases of fever; consequently, the poliomyelitis cases reported from Finnish rural districts are almost exclusively paralytic. Only in epidemic years are non-paralytic cases more frequently diagnosed and reported.

Table 1 shows the poliomyelitis morbidity per 100,000 members of the population between 1905 and 1954, calculated from all poliomyelitis cases reported, and related to the population figures for each year. The morbidities from the years 1905—38 are taken from Halminen (49). The reports for 1940—42 must be considered incomplete on account of the war.

TABLE 1

ANNUAL RATES PER 100,000 INHABITANTS OF REPORTED CASES OF POLIOMYELITIS IN FINLAND, 1905—54

| Year | Rates per 100,000 | Mean value |
|---|---|---|
| 1905 [1] | 1.3 | 1.0 |
| 1906 | 0.7 | |
| 1911 | 8.4 | 2.0 |
| 1912 | 4.3 | |
| 1913 | 1.2 | |
| 1914 | 0.8 | |
| 1915 | 0.8 | |
| 1916 | 1.2 | |
| 1917 | 0.4 | |
| 1918 | 0.4 | |
| 1919 | 0.4 | |
| 1920 | 0.4 | |
| 1921 | 0.4 | |
| 1922 | 0.8 | |
| 1923 | 1.3 | |
| 1924 | 1.3 | 1.0 |
| 1925 | 0.8 | |
| 1926 | 0.4 | |
| 1927 | 1.7 | |
| 1928 | 0.7 | |
| 1929 | 2.9 | |
| 1930 | 7.5 | 6.5 |
| 1931 | 2.7 | |
| 1932 | 8.5 | |
| 1933 | 3.7 | |
| 1934 | 11.6 | |
| 1935 | 5.0 | |
| 1936 | 2.9 | |
| 1937 | 5.4 | |
| 1938 | 16.0 | |
| 1939 [2] | 2.2 | |
| 1940 | 14.7 | 7.7 |
| 1941 | 2.1 | |
| 1942 | 1.1 | |
| 1943 | 5.8 | |
| 1944 | 10.4 | |
| 1945 | 21.5 | |
| 1946 | 6.6 | |
| 1947 | 5.9 | |
| 1948 | 2.5 | |
| 1949 | 6.1 | |
| 1950 | 8.0 | 8.0 |
| 1951 | 3.7 | |
| 1952 | 2.0 | |
| 1953 | 7.6 | |
| 1954 | 18.8 | |

[1] 1905—38 according to Halminen.
[2] 1939—54 according to the State Medical Board.

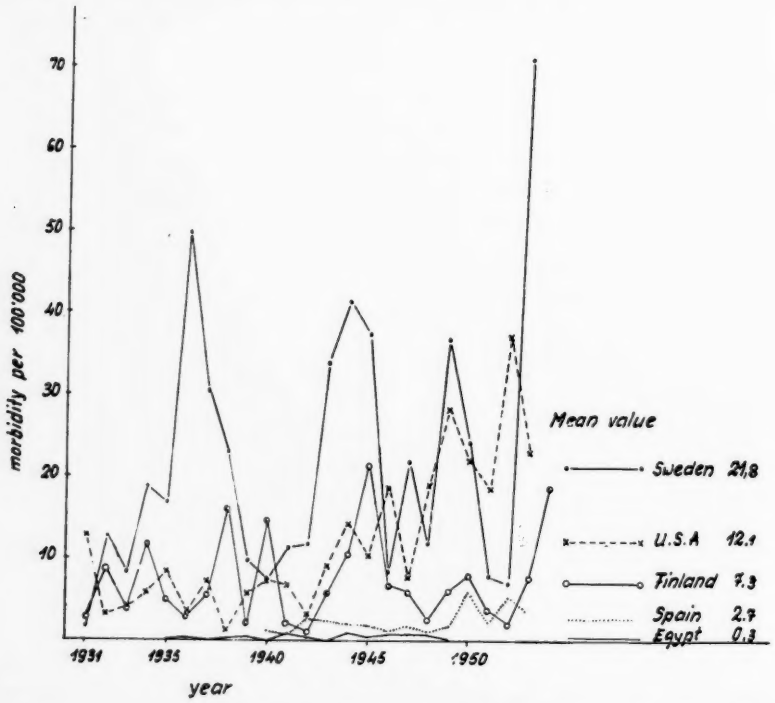


Fig. 1. — Annual rates per 100,000 inhabitants of reported cases of poliomyelitis in Finland (136), Sweden (40), U.S.A. (40), Spain (40) and Egypt (126), 1931—53.

A study of Table 1 will show that the morbidity varies greatly from year to year. 1911 and 1912 are epidemic years. After that, the morbidity remains low, less than 1.0 on an average, until it begins to increase in the 1930's. From then on it varies between a minimum of 1.1 and a maximum of 21.5, exceeding the average about every 3 to 5 years; only in 1945 did the morbidity exceed 20. The mean morbidity has been calculated for each decade as an arithmetic mean of the yearly morbidities. It is 1.0—2.0 in the beginning, but reaches 6.5 in the 1930's, and then rises to 7.7 in the 1940's and to 8.0 in the 1950's. It can thus be concluded that the morbidity has gradually increased.

Fig. 1 shows a comparison between the morbidity curve for the years 1931—53 calculated from all the poliomyelitis cases reported in Finland, Sweden (40), U.S.A. (40), Spain (40) and Egypt (126). The mean morbidity for these years is:

| | | | | |
|---|---|---|---|---|
| Sweden ..... | 21.8 | per | 100,000 | inhabitants |
| U.S.A. ..... | 12.1 | » | » | » |
| Finland ..... | 7.3 | » | » | » |
| Spain ..... | 2.7 | » | » | » |
| Egypt ..... | 0.3 | » | » | » |

The figure demonstrates the high morbidity in Sweden, the increase of the morbidity in the U.S.A. in recent years, and, in contrast, the low morbidities of Spain and Egypt. The great difference between the morbidity figures of two such close geographical neighbours as Sweden and Finland is also remarkable, those for Sweden being on an average three times as high as those for Finland. The epidemics in Sweden have occurred at somewhat longer intervals than in Finland. So far, Finland has been spared very severe epidemics.

THE INCIDENCE OF POLIOMYELITIS IN VARIOUS PROVINCES

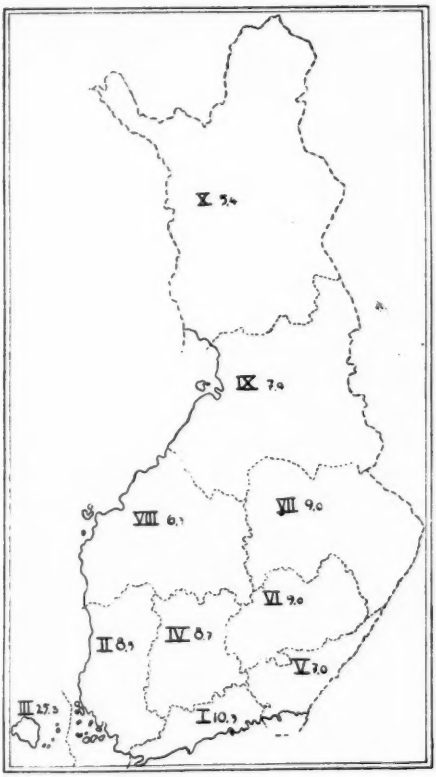


Fig. 2. — Annual mean morbidity of reported poliomyelitis cases in years 1943—54 in the provinces of Finland.

Administratively, Finland is divided into 10 provinces, which will be referred to in the following by the Roman numerals given in Fig. 2. The accompanying figures indicate the annual mean morbidity for each province for the period 1943—54, the annual mean of reported cases in each province during this time being related to its mean population.

Only for provinces III and X are the deviations shown by the mean morbidities statistically significant. The peculiar geographical position of province III may account for its deviating morbidity (109), whereas, in the case of province X (Lapland) the low morbidity may be explained either as resulting from the smallness and wide dispersal of the population which provides too few contacts for the sustaining of an epidemic, or by assuming that less severe cases do not come under the physicians' notice on account of the great distances.

With the exception of these slight differences, attributable to

TABLE 2

ANNUAL RATES PER 100,000 INHABITANTS OF REPORTED CASES OF POLIOMYELITIS IN THE DIFFERENT PROVINCES, 1943—54

| Year | I Uusimaa 710.2[1] | II Turun ja Porin lääni 641.8 | III Ahvenanmaa 21.4 | IV Hämeen lääni 572.5 | V Kymin lääni 318.9 | VI Mikkelin lääni 245.9 | VII Kuopion lääni 482.4 | VIII Vaasan lääni 617.5 | IX Oulun lääni 378.0 | X Lapin lääni 176.7 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1943 | 5.5 | 3.5 | — | 5.0 | 1.7 | 2.0 | 20.4 | 2.5 | 7.5 | 5.0 |
| 1944 | 15.7 | 7.6 | 32.4 | 9.6 | 4.2 | 12.5 | 13.3 | 11.7 | 7.4 | 9.2 |
| 1945 | 21.4 | 46.2 | 4.6 | 28.9 | 28.2 | 16.7 | 16.6 | 12.8 | 13.9 | 5.0 |
| 1946 | 6.9 | 8.2 | — | 7.8 | 1.6 | 6.2 | 8.8 | 7.8 | 8.1 | 2.7 |
| 1947 | 6.2 | 4.9 | — | 8.9 | 6.2 | 7.4 | 5.3 | 6.9 | 6.7 | 1.3 |
| 1948 | 2.6 | 2.7 | — | 2.3 | 2.7 | 3.6 | 3.3 | 1.2 | 3.3 | 5.1 |
| 1949 | 7.3 | 6.7 | — | 6.2 | 5.2 | 4.2 | 2.9 | 5.0 | 12.1 | 5.5 |
| 1950 | 11.1 | 6.8 | 4.6 | 9.2 | 6.1 | 8.3 | 7.7 | 6.4 | 7.2 | 7.8 |
| 1951 | 2.6 | 3.3 | — | 4.5 | 0.3 | 3.7 | 5.1 | 3.6 | 4.1 | 8.9 |
| 1952 | 1.9 | 3.3 | — | 2.5 | 2.2 | 1.2 | 2.5 | 1.3 | 0.5 | 1.2 |
| 1953 | 4.5 | 4.4 | 261.7 | 7.0 | 1.9 | 3.7 | 12.7 | 4.9 | 12.7 | 3.4 |
| 1954 | 36.4 | 8.1 | — | 15.4 | 32.5 | 36.8 | 11.7 | 11.9 | 11.5 | 9.6 |
| Mean value[2] | 10.3 | 8.5 | 25.3 | 8.7 | 7.0 | 9.0 | 9.0 | 6.3 | 7.9 | 5.4 |

[1] whole population in 1954 (thousands).

[2] mean value of poliomyelitis cases per 100,000 members of annual mean population.

the geographical location of the provinces, it can be said that the incidence of poliomyelitis is roughly equal in the different provinces of Finland.

Detailed data on the poliomyelitis morbidity per 100,000 members of the population in the different provinces of Finland during the period 1943—54 are given in Table 2.*

## THE INCIDENCE OF POLIOMYELITIS IN URBAN AND IN RURAL DISTRICTS

Table 3 shows detailed data of the morbidities and the mean poliomyelitis morbidity per 100,000 members of the population during the period 1943—54 in rural districts, in small cities and in cities with over 100,000 inhabitants. The mean morbidities have been calculated from the mean of all reported poliomyelitis cases during this period and from the mean populations.

TABLE 3

NUMBER OF CASES OF POLIOMYELITIS REPORTED AND CASE RATE PER 100 000 INHABITANTS IN CITIES AND IN RURAL DISTRICTS, 1943—54

| Year | Cities: Population >100,000 | | | Small Cities | | | Rural Districts | | |
|---|---|---|---|---|---|---|---|---|---|
| | No of cases | Population (in thousands) | Rates per 100,000 | No of cases | Population (in thousands) | Rates per 100,000 | No of cases | Population (in thousands) | Rates per 100,000 |
| 1943 | 24 | 422.0 | 5.7 | 30 | 397.7 | 7.5 | 156 | 2.855.4 | 5.5 |
| 1944 | 62 | 440.2 | 14.1 | 42 | 379.7 | 11.1 | 278 | 2,873.4 | 9.7 |
| 1945 | 102 | 515.0 | 19.8 | 62 | 387.3 | 16.0 | 630 | 2,835.4 | 22.2 |
| 1946 | 40 | 545.6 | 7.3 | 26 | 410.0 | 6.3 | 182 | 2,892.8 | 6.3 |
| 1947 | 39 | 547.7 | 7.1 | 28 | 430.0 | 6.5 | 161 | 2,932.9 | 5.5 |
| 1948 | 13 | 554.8 | 2.3 | 9 | 440.9 | 2.0 | 76 | 2,962.8 | 2.6 |
| 1949 | 50 | 568.1 | 8.8 | 24 | 456.6 | 5.3 | 167 | 2.982.4 | 5.6 |
| 1950 | 75 | 582.8 | 12.9 | 30 | 473.8 | 6.3 | 217 | 3,020.0 | 7.2 |
| 1951 | 33 | 595.6 | 5.5 | 17 | 483.2 | 3.5 | 100 | 3,043.0 | 3.3 |
| 1952 | 28 | 607.1 | 4.6 | 11 | 493.0 | 2.2 | 43 | 3,070.2 | 1.4 |
| 1953 | 32 | 611.4 | 5.2 | 30 | 522.0 | 6.0 | 254 | 3,070.3 | 8.3 |
| 1954 | 177 | 624.0 | 28.4 | 101 | 535.7 | 20.0 | 512 | 3,089.1 | 16.6 |
| Mean value | 56.3 | 550.7 | ***10.1*** [1] | 34.2 | 450.8 | ***7.6*** [1] | 231.3 | 2,969.0 | ***7.8*** [1] |

[1] mean value of poliomyelitis cases per 100,000 members of annual mean population.

* A report of poliomyelitis in Finland 1953—55 was published by Lojander and Pätiälä (90).

It will be seen from this table that the morbidity is equal in rural districts and in small cities, and higher in cities with more than 100,000 inhabitants. The difference is statistically significant.

The statistical difference in morbidity between the large cities and the rural areas may be real, but may equally well be caused by the fact that nonparalytic poliomyelitis cases come more frequently to the physicians' notice in large cities. Support is lent to this assumption by the 1954 report on poliomyelitis in the city of Helsinki, which shows nonparalytic poliomyelitis for 54 cases out of 153, i.e. 35%, whereas only 121 of the 637 cases reported from elsewhere in Finland, i.e. only 19%, were nonparalytic and unspecified cases.

In Helsinki all poliomyelitis patients are treated at the Aurora, hospital for infectious diseases, and the paralytic cases can thus be separated by means of the case histories. The mean morbidity for the period 1943—54 calculated from paralytic cases is 8.2. This figure, 8.2, is not quite comparable with the mean morbidity from all poliomyelitis cases of the rural areas, 7.8 and 7.6, but, considering the rare reporting of nonparalytic cases from the rural areas, it may be said that no remarkable difference exists between the morbidities in the cities and in the rural districts.

### THE INCIDENCE OF POLIOMYELITIS BY AGE GROUPS

The ages of the poliomyelitis patients are not given in the reports on infectious diseases sent to the State Medical Board during the period 1939—52. Consequently, the distribution according to age groups could only be studied in the 1953 and 1954 material.

Fig. 3 shows the incidence of poliomyelitis in Finland in 5-year groups in the epidemic year 1954. The dark columns represent the paralytic and the white columns the nonparalytic cases. Altogether 790 cases were observed in 1954, of which 615 were paralytic, but as the ages of 48 patients (30 nonparalytic and 18 paralytic) are unknown, the material used for Fig. 3 comprises only 742 cases.

A glance at this figure will show that the greater part of the nonparalytic cases occur in the younger groups, mainly in that of children under 10 years. It is well-known that the manifestation of virus infections is less severe in children than in adults. Children

under 5 years constitute the largest group of patients in the Finnish material, while the subsequent five-year groups show a gradual decrease, until in the two groups between 20 and 30 years the number of cases once more exceeds that of the 15—19 year-old group. After this the number of cases falls abruptly, and poliomyelitis occurs very rarely after 40 years of age in Finland.

The increase in morbidity among persons between 20 and 30 years might have been caused by the differences in number between the population groups concerned. To further elucidate this question, the morbidity curves per 100,000 members of the population in each group as calculated separately from the paralytic cases and from all reported cases according to the mean population of each age group in 1954, have been plotted in the figure. This method of computation, too, shows that the morbidity is highest in children under five years and reaches a new peak in the 20—30 year-old groups.

Detailed data on the number of cases, on the morbidity and on the percentual distribution among the various age groups in 1953 and 1954 are given in Table 4.

TABLE 4

MORBIDITY AND PERCENTUAL DISTRIBUTION OF POLIOMYELITIS CASES IN 5-YEAR AGE GROUPS 1953 AND 1954 IN FINLAND

| Age group | 1953 | | | 1954 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | All cases | | | All cases | | | Paralytic cases | | |
| | Number | Rates per 100,000 | Per cent | Number | Rates per 100,000 | Per cent | Number | Rates per 100,000 | Per cent |
| 0— 4 | 66 | 14.1 | 24.9 | 190 | 41.7 | 25.6 | 156 | 34.3 | 26.1 |
| 5— 9 | 55 | 12.1 | 20.8 | 184 | 38.2 | 24.8 | 125 | 26.1 | 20.9 |
| 10—14 | 31 | 9.1 | 11.7 | 87 | 25.4 | 11.7 | 71 | 20.7 | 11.9 |
| 15—19 | 34 | 10.8 | 12.8 | 55 | 16.9 | 7.4 | 47 | 14.4 | 7.9 |
| 20—24 | 18 | 5.7 | 6.8 | 66 | 21.2 | 8.9 | 59 | 19.0 | 9.9 |
| 25—29 | 22 | 7.0 | 8.3 | 75 | 23.8 | 10.1 | 66 | 21.0 | 11.0 |
| 30—34 | 14 | 5.0 | 5.3 | 48 | 16.4 | 6.5 | 39 | 13.3 | 6.5 |
| 35—39 | 12 | 4.5 | 4.5 | 17 | 6.5 | 2.3 | 15 | 5.7 | 2.5 |
| ≥40 | 13 | 0.9 | 4.9 | 20 | 1.4 | 2.7 | 20 | 1.4 | 3.3 |
| unspecified | 51 | • • | • | 48 | • • | • | 18 | • • | • |
| Total | 316 | • • | 100.0 | 790 | • • | 100.0 | 615 | • • | 100.0 |

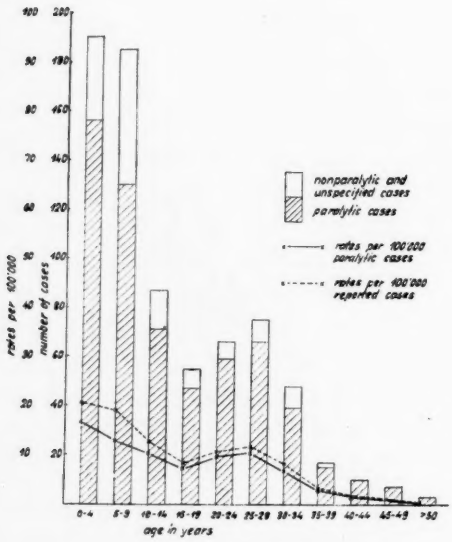
nonparalytic and unspecified cases
paralytic cases
rates per 100 000 paralytic cases
rates per 100 000 reported cases
rates per 100 000
number of cases
age in years

Fig. 3. — Poliomyelitis cases and morbidity per 100,000 inhabitants in 5-year groups in the epidemic year 1954.

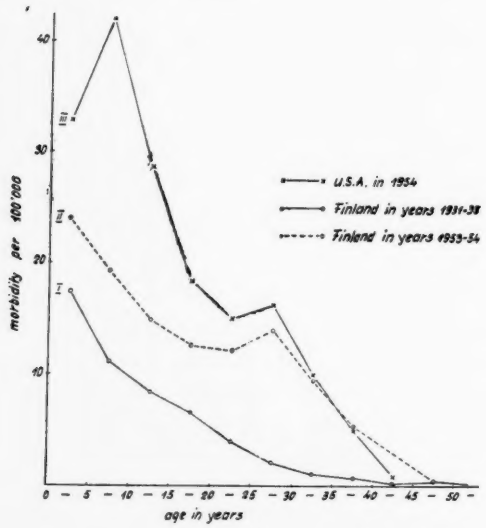


Fig. 4. — Annual mean morbidity of reported poliomyelitis cases per 100,000 inhabitants in 5-year groups in Finland 1931—38 (49), 1953—54 and in U.S.A. 1954 (133).

Fig. 4 shows the morbidity curves for Finland, calculated in five-year age groups from all reported poliomyelitis cases, for the periods 1931—38 and 1953—54, compared with the corresponding curve per 100,000 members of the population for the U.S.A. in the year 1954. It will be seen that the Finnish curve for 1953—1954 has a different shape from that for 1931—38 particularly with regard to persons between 20 and 30 years of age. One cannot speak of an increase in morbidity on the basis of these curves, since the higher than normal values in the 1953—54 curve are due to the 1954 epidemic. The curve for the U.S.A. shows a similar peak in the 20—30 year group. The curves differ in that in Finland the highest morbidity occurs in the group of children under five, whereas in the U.S.A. it comes in the 5—9 year group. During the 1953 poliomyelitis epidemic in Sweden, the morbidity curve calculated from the paralytic cases for the different age groups was found to approximate the shape of the U.S.A. curve, i.e. with the highest morbidity in the 5—9 year group and a distinct rise in the morbidity of persons between 20 and 35 years of age (113).

TABLE 5

PERCENTUAL AGE DISTRIBUTION OF POLIOMYELITIS CASES IN FINLAND, 1931—54

| Age groups | 1931 | 1933 | 1937 | 1938 | 1953 | 1954 | |
|---|---|---|---|---|---|---|---|
| | All cases (according to Halminen) | | | | All cases | All cases | Paralytic |
| 0— 4 | 36.4 | 37.9 | 23.9 | 17.4 | 24.9 | 25.6 | 26.1 |
| 5— 9 | 25.2 | 17.4 | 26.8 | 26.5 | 20.8 | 24.8 | 20.9 |
| 10—14 | 17.2 | 18.2 | 21.0 | 19.6 | 11.7 | 11.7 | 11.9 |
| 15—19 | 9.1 | 12.5 | 12.7 | 18.1 | 12.8 | 7.4 | 7.9 |
| ≥20 | 12.1 | 14.0 | 15.6 | 18.4 | 29.8 | 30.5 | 33.2 |
| | *21.2* | *26.5* | *28.3* | *36.5* | *42.6* | *37.9* | *41.1* |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

Table 5 shows the percentage of the reported poliomyelitis cases in different age groups during the period 1931—54. The percentual figures for 1931—38 are taken from Halminen (49). The last column of the table gives the percentual distribution of paralytic poliomyelitis cases alone in 1954.

As has been seen from the morbidity curve, the group of patients under 5 years was the largest group in Finland in 1953 and 1954. The total percentage of persons over 15 years, given in each column, shows how the percentage of adults has increased, particularly

among persons over 20 years of age. Thus, the shift of the poliomyelitis disease towards older age groups which has been established in other countries is also observable in Finland.

For comparison the percentual distribution according to age group of the poliomyelitis cases in Egypt (125), Spain (40), the U.S.A. (133), Finland and Sweden (113) is shown in Table 6.

TABLE 6

PERCENTUAL DISTRIBUTION ACCORDING TO AGE OF THE POLIOMYELITIS CASES REPORTED IN DIFFERENT COUNTRIES

| Age group | Egypt [1] 1948—49 | Spain [2] 1952 | U.S.A. [3] 1954 | Finland 1954 | Sweden [4] 1953 |
|---|---|---|---|---|---|
| <1 | 30.1 | 28.2 | | | |
| 1— 2 | 60.7 | 50.5 | 24.8 | 25.6 | 14.0 |
| 3— 4 | 8.9 | 12.7 | | | |
| 5— 6 | — | 3.8 | 27.3 | 24.8 | 19.0 |
| 7— 8 | — | 2.3 | | | |
| 9—10 | — | 1.5 | | | |
| 10—14 | — | | 15.0 | 11.7 | 9.6 |
| 15—19 | 0.3 | 1.0 | 8.4 | 7.4 | 8.9 |
| over 20 | — | | 24.5 | 30.5 | 48.4 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

[1] (125), [2] (40), [3] (133), [4] Calculated from paralytic poliomyelitis cases (113).

It will be seen from this table that in Egypt nearly 100% and in Spain over 90% of all poliomyelitis cases occur in children under five years of age. The proportional distribution of poliomyelitis cases in Finland in 1954 was very similar to that in the U.S.A., about one quarter of the cases occurring in children under 5 and half of the cases in children under 10 years. In Sweden the proportion of adults was higher, about half of the cases occurring in persons over 20 years of age.

## THE INCIDENCE OF POLIOMYELITIS IN HELSINKI

In Helsinki information is available on the paralytic poliomyelitis cases. In addition, the occurrence of the neutralizing antibodies against poliomyelitis virus has been investigated in Helsinki. For this reason the incidence of clinical poliomyelitis in Helsinki will be treated separately.

The following tables and figures relating to the inhabitants of Helsinki have been compiled from paralytic cases only.

*The Morbidity in Helsinki*

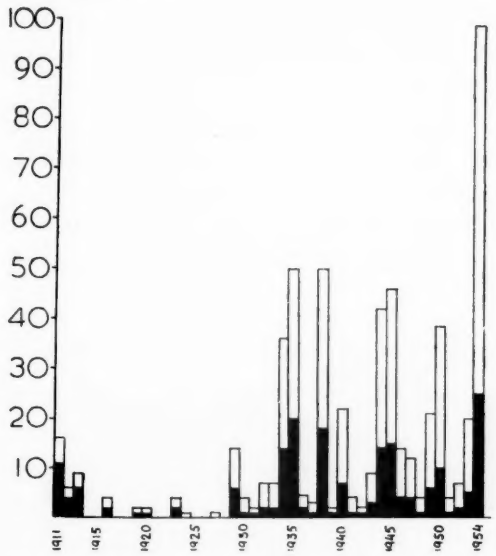


Fig. 5. — Paralytic poliomyelitis cases in Helsinki, 1911—54. The whole column represents the number of paralytic cases and the black part the morbidity per 100,000 inhabitants.

[Reproduced from O. Pettay (130)]

Fig. 5 shows the annual incidence of paralytic poliomyelitis and the annual morbidity per 100,000 inhabitants in the years 1911—54. (130). It will be seen that the incidence of poliomyelitis increased considerably from 1930 onwards. The worst epidemic to occur in Helsinki was in 1954; poliomyelitis has also occurred more frequently than normal in 1935, 1938, 1944 and 1945.

*The Incidence of Paralytic Poliomyelitis by Sex*

From 1944 onwards more detailed investigations have been made on the paralytic poliomyelitis cases occurring in Helsinki. Altogether 320 cases occurred during the period 1944—54, of which 99 came in 1954, the epidemic year (104 cases according to the report, corrected to 99 on the basis of the case histories).

The number of females was 145, or 45%, and that of males 175, or 55% of all cases. The difference becomes even more pronounced, if the greater proportion of females in the population is taken into account. During this 11-year period there occurred 64.7 poliomyelitis cases per 100,000 females and 103.2 cases per 100,000 males. In this material the number of males in various age groups has been higher than that of females with the exception of the age groups 5—14 (Table 7).

A greater susceptibility to paralytic poliomyelitis among males has also been observed in other countries (40). According to statistics from Stockholm, 60% of the paralytic cases in the 1953 epidemic were males and 40% females; the percentage of males was higher than that of females in every age group (27).

### *The Incidence of Paralytic Poliomyelitis by Age*

The age distribution of the paralytic poliomyelitis cases in Helsinki during the period 1944—55 (320 cases) is given in Table 7. This table gives the annual morbidity per 100,000 members of the population in five-year groups for persons under 20 and in ten-year groups for persons over 20 years. The distribution is shown graphically in Fig. 6.

TABLE 7

NUMBER OF CASES AND MEAN ANNUAL MORBIDITY OF PARALYTIC POLIOMYELITIS PER 100,000 MEMBERS OF THE POPULATION IN HELSINKI IN 1944—54 AND IN STOCKHOLM IN 1953 (27)

| Age group | Paralytic cases in Helsinki 1944—54 | | | | | Annual morbidity per 100,000 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ♂ | | ♀ | | Total | Helsinki | | | Stockholm 1953 |
| | No | % | No | % | | 1944—54 | 1944—53 | 1954 | |
| 0— 4 | 46 | 26 | 30 | 21 | 76 | 22.1 | 16.0 | 82.8 | 162 |
| 5— 9 | 18 | 10 | 21 | 15 | 39 | 13.9 | 7.8 | 54.6 | 200 |
| 10—14 | 11 | 6 | 16 | 11 | 27 | 13.3 | 10.7 | 31.7 | 79 |
| 15—19 | 28 | 16 | 20 | 14 | 48 | 18.0 | 19.9 | 48.8 | 78 |
| 20—29 | 43 | 25 | 34 | 23 | 77 | 9.2 | 10.1 | 31.8 | 120 |
| 30—39 | 20 | 11 | 20 | 14 | 40 | 4.4 | 4.8 | 14.2 | 93 |
| 40—49 | 8 | 5 | 4 | 3 | 12 | 1.1 | 1.2 | 10.5 | 22 |
| ≥50 | 1 | 1 | | | 1 | 0.1 | — | 1.0 | 3 |
| Total | 175 | 100 | 145 | 100 | 320 | . . | . . | . . | . . |

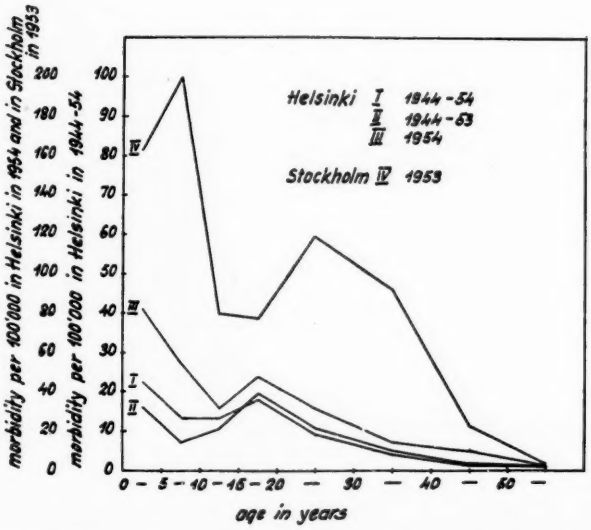
morbidity per 100 000 in Helsinki in 1954 and in Stockholm in 1953
morbidity per 100 000 in Helsinki in 1944-54
Helsinki I 1944-54
II 1944-53
III 1954
Stockholm IV 1953
0 - 5 - 10 - 15 - 20 — 30 — 40 — 60 —
age in years

Fig. 6. — Mean annual morbidity of paralytic poliomyelitis cases per 100,000 inhabitants in 5-year groups in Helsinki in 1944—54 and in Stockholm in 1953 (27).

As can be observed from the table and the figure, during the period 1944—54 the morbidity was highest in the group of children under 5 years, decreased in the following age groups, only to reach a new peak in the 15—19 year group and then fall abruptly after the age of 40 years.

The morbidity figures for the epidemic year 1954 show a similar increase in the 15—19 year group. In order to eliminate the influence of the increase caused by the epidemic, the annual morbidity per 100,000 members of the population was calculated for the 1944—53 period alone, but the general shape of the curve remained unchanged.

In order to compare the poliomyelitis incidences in Helsinki and Stockholm during the epidemic years, the corresponding figures for Stockholm have been included in both the table and the figure (27). A study of the morbidity curves for these epidemic years will draw particular attention to the differences in the height of the morbidity figures and in the positions of the peaks. The first peak, occurring in Helsinki in children under 5 years, appears in Stockholm in the 5—9 year group, while the second peak comes between 15 and 19 years in Helsinki and between 20 and 30 in Stockholm.

TABLE 8

PERCENTUAL DISTRIBUTION OF THE PARALYTIC POLIOMYELITIS CASES, ACCORDING TO AGE GROUP, IN HELSINKI 1944—54 AND IN STOCKHOLM 1953 (27)

| Age group | Helsinki | | Stockholm |
|---|---|---|---|
| | 1944—53 | 1954 | 1953 |
| 0— 4 | 22.6 | 26.0 | 40.1 |
| 5— 9 | 8.6 | 20.0 | |
| 10—14 | 8.6 | 8.0 | 7.0 |
| 15—19 | 17.2 | 10.0 | 5.2 |
| 20—29 | 25.9 | 20.0 | 21.0 |
| 30—39 | 14.0 | 9.0 | 20.0 |
| 40—49 | 3.2 | 5.0 | 6.7 |
| over 50 | — | 1.0 | |
| | 60.3 | 45.0 | 52.9 |
| Total | 100.0 | 100.0 | 100.0 |

Table 8 shows the percentage of poliomyelitis cases belonging to the different age groups in Helsinki during the period 1944—54 and in Stockholm in 1953.

Particularly noticeable is the high percentage of paralytic poliomyelitis cases in Helsinki among persons over 15 years: it was as high as 60% in 1944—53 and 45% in the epidemic year 1954; in Stockholm it was 53% in the epidemic year 1953 (27).

*The Incidence of Paralytic Poliomyelitis in Different Social Classes*

In Helsinki no great differences exist in the modes of life of people from different social classes. Their division into social classes on a professional basis is bound to be rather inaccurate, yet an attempt has been made to divide the poliomyelitis patients of the period 1944—54 into four social classes, using the social classification based on profession which is recommended by the Statistical Office of Helsinki. The resulting distribution of the cases is shown in Table 9.

Of the paralytic poliomyelitis cases from the period 1944—54, about 45% belong to the higher income classes I and II. Since about 40% of the population of Helsinki belongs to these classes (165), no distinct difference is observable in the incidence of the poliomyelitis cases investigated in the different income brackets.

TABLE 9

AGE DISTRIBUTION OF PARALYTIC POLIOMYELITIS CASES IN DIFFERENT SOCIAL CLASSES IN HELSINKI, 1944—54

| Social class | Age, years | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0—4 | 5—9 | 10—14 | 15—19 | 20—29 | 30—39 | 40—49 | >50 | | |
| I (highest incomes) | 23 | 14 | 8 | 7 | 21 | 11 | 4 | 1 | 89 | 145 |
| II | 16 | 5 | 3 | 6 | 19 | 6 | 1 | — | 56 | |
| III | 31 | 15 | 12 | 12 | 26 | 13 | 4 | — | 113 | 175 |
| IV | 6 | 5 | 4 | 23 | 11 | 10 | 3 | — | 62 | |
| Total | 76 | 39 | 27 | 48 | 77 | 40 | 12 | 1 | 320 | |

A significant feature of Table 9 is the high morbidity of persons between 15 and 19 years in the lowest social class. This is due to the fact that many persons of this age are at the apprentice stage and are therefore included in the lowest income class.

## SUMMARY OF THE EPIDEMIOLOGICAL PATTERNS

*In Finland* statistics on the poliomyelitis disease have been kept since 1905. Its occurrence has been more frequent since 1930. Unfortunately, before the autumn of 1953 no distinction was made on the reports between paralytic and nonparalytic cases. Therefore calculations are based on all poliomyelitis cases reported.

The annual morbidity per 100,000 members of the population has increased gradually from 6.5 in the 1930's to 7.7 in the 1940's and 8.0 in the 1950's. No particularly severe epidemics have occurred; the worst epidemics were those of 1945 and 1954 with a morbidity per 100,000 members of the population of 21.5 and 18.8, respectively, calculated on the basis of all reported cases.

The disease has a uniform distribution in various provinces with the exception of provinces III and X (Fig. 2), whose geographic location is exceptional. No remarkable difference in morbidity is observable between the cities and the rural districts.

Poliomyelitis statistics indicate that a shift has occurred towards the older age groups. In the 1930's the proportion of persons over 15 years with notified poliomyelitis was 21—28% and during the period 1953—54 about 40%. The annual morbidity per 100,000 members of the population is greatest in the group of children under 5 years. The figures then fall until the 15—19-year-old age group is

reached, rising again through the 20—29-year-old group after which there is an abrupt decline, very few persons over 40 years having poliomyelitis.

In *Helsinki* information was available on the paralytic poliomyelitis cases. Accordingly, all calculations from that city are based on paralytic poliomyelitis cases. In Helsinki, as in the entire country, poliomyelitis has been considerably more frequent since 1930. The annual morbidity during the last ten years has been 8.2 per 100,000 inhabitants. The most severe epidemic occurred in 1954, when the morbidity reached 25 per 100,000 members of the population, taking into account only the paralytic cases.

In the age distribution the under 5-year-old group has the highest percentage of poliomyelitis cases. In the following groups the morbidity falls, only to rise again in the 15—19 year-old group. After that a slow decrease sets in and persons over 50 years contract poliomyelitis only rarely. The percentage of persons over 15 years is remarkably high: 60% during the period 1944—53 and 45% in the epidemic year 1954. No difference in morbidity can be observed between the different social classes during the period 1944—54.

Paralytic poliomyelitis has occurred more frequently in males than in females, the morbidities for the period 1944—54 being 103 per 100,000 males and 65 per 100,000 females.

## THE OCCURRENCE OF POLIOMYELITIS NEUTRALIZING ANTIBODIES IN FINLAND

According to the literature, the presence of neutralizing antibodies to poliomyelitis is a sign of previous infection, either clinical or subclinical. Estimates of the ratio of paralytic cases to inapparent poliomyelitis infections vary within a considerable range. However, such estimates as 1 in 100 or 1 in 1000 (v. p. 90) indicate that an overwhelming majority of mankind undergo poliomyelitis on a subclinical level.

The aim of the present work was to establish the distribution of poliomyelitis infection in various age groups in certain districts in Finland by determining the occurrence of neutralizing antibodies against the three types of poliomyelitis virus in the sera of healthy persons.

## MATERIAL

Blood samples were collected from 2,111 Finns during the period January to July, 1954, i.e. before the poliomyelitis epidemic which began in August 1954.

*City dwellers* were represented by 1,084 inhabitants of the capital, Helsinki, whose blood samples were obtained as follows:

The sera of adults from Helsinki were collected at the Red Cross Blood Service in January, 1954, those of children under 15 years at the Aurora Hospital (Municipal Hospital for children and infectious diseases) during the period February to May 1954, except for the samples from children under 2 years which were obtained in July 1954. The 75 sera obtained from Docent K. Penttinen, M.D., mainly from persons between 10 and 14 years of age, were taken in June 1954 before the vaccination against poliomyelitis. The sera of persons between 15 and 19 years were mainly obtained in July 1954. Most of them belonged to the staff of a department store.

In *rural districts*, public health assistants collected 1,027 blood samples from volunteers of varying ages. Eastern Finland is represented by the rural region around the city of Joensuu, from which 306 samples were obtained. In Northern Finland, 169 blood samples were collected in the communes of Kemijärvi and Sodankylä. In Central Finland the communes of Padasjoki, Luopioinen and Kuhmoinen were chosen because in these districts, according to the reports sent to the State Medical Board, poliomyelitis cases have occurred comparatively rarely and in small numbers: 248 blood samples were collected in this area. All the samples mentioned had been collected before the end of July, 1954. Since the antibody status in Central Finland differed from that in the other districts investigated, another series of 304 blood samples was collected in January 1955 from the same communes in Central Finland. This time proportional sampling was used, i.e. samples were taken in each 5-year group in every village, even outlying ones, in proportion to the population of the villages.

The samples, taken under sterile conditions, were mailed from the rural districts to the laboratory, where they were immediately centrifuged at 2000 r.p.m. for 20 minutes. The serum was separated, inactivated at 56°C for ½ hour and stored in the refrigerator at + 4°C.

*Viruses.* — The viruses used in the neutralization test were obtained through the courtesy of Dr. Tore Wesslen, M.D. of the State Bacteriological Laboratory, Stockholm, Sweden. Type I was a Brunhilde strain, type II the MEF1 strain, and type III the Leon strain. They were transported to Helsinki in dry ice-cooled containers and were stored in the laboratory at —15 to —20°C. Their titre on arrival was: type I $10^{-6}$, type II $10^{-5}$, type III $10^{-6}$.

## METHODS

The determinations of the antibodies to the three different types of poliomyelitis were made by a neutralization test in fixed roller tube cultures of fibroblasts from human embryonic and fetal lung tissue. The undiluted sera were tested against an estimated 100 $TCD_{50}$ of virus. 100 $TCD_{50}$ = 100 tissue culture doses with 50% endpoint according to Reed and Muench (135).

The method with human embryonic fibroblasts in roller-tube-system was chosen, because at the time this work was started there did not seem to be any possibility for other types of tissue culture in this country. In addition this method had already been adopted in Sweden, where the author had the opportunity of studying tissue culture technique with the intention of introducing the method in Finland.

### *Preparation of the Tissue Cultures*

#### *Glassware and Other Apparatus Required*

Pyrex glassware was employed except for the Pasteur pipettes.

The *tissue culture tubes*, 15 × 100 mm in size, were cleaned with extreme care. Contaminated tubes were immediately autoclaved at 120°C for ½ hour. While still hot, the tubes were washed several times with a brush in hot water to which 15 $cm^3$ of »Calgonite» per 10 litres of water (a washing powder containing sodium hexametaphosphate, manufactured by Allbright & Willman) had been added. The tubes were immediately rinsed individually in 10 changes of hot tap water, and then in 10 changes of cold tap water. After this they were boiled in 1% hydrochloric acid for one hour. While still hot, they were once more carefully rinsed in about 10—15 changes of hot tap water and then 10—15 times in cold water. All the washing was done with tap water, i.e. without the use of distilled

water. The tubes were finally dried in hot air at 150°C and sterilized for one hour at 150—160°C.

New, previously unused glass tubes were placed in 25% sulphuric acid for one hour, rinsed ten times in tap water, and then treated as described above.

The *pipettes* were immersed overnight in chloramin solution. They were washed several times with suction in Calgonite water and rinsed about 10 times in tap water. After this they were dried and sterilized for one hour in hot air at 150—160°C. They were stored in a sterile cover for not more than one or two days.

The *Pasteur pipettes* were made of soft glass tubing, 8 mm in diameter, and were washed in the same manner as the ordinary glassware. The Pasteur pipettes required were sterilized in the same manner as the other pipettes, on the day on which they were used. They were used once and discarded.

The *stoppers* were black rubber stoppers of domestic make. After use they were autoclaved for ½ hour at 120°C., were then scrubbed and washed in hot Calgonite water and rinsed in many changes of hot and cold tap water. They were dried in a towel and sterilized in the autoclave at 120°C for ½ hour. New, previously unused rubber stoppers were boiled for 15 minutes in 0.5 *N* NaOH solution, rinsed several times in tap water, boiled in 4% hydrochloric acid and rinsed again many times in tap water. After this they were treated in the same way as the used stoppers.

Four *rotary drums* were mounted in an *incubator* kept at 37°C. The four drums which rotated at 30 revolutions per hour, had a capacity of 1,100 culture tubes.

*Preparation of Tissue.* — The *tissue* used in the tests consisted of fibroblasts from human embryonic and fetal lungs. The embryos were obtained from the maternity hospitals in Helsinki, most of them from the Women's Clinic of Helsinki University from quite recently performed minor caesarian section operations. The embryos were usually in the amniotic sac and ranged in age between the II and V intra-uterine months. The lungs were removed from the embryo not later than two hours after the operation, surgical asepsis being observed. Since the tissues of younger embryos appeared to proliferate better than those of older fetuses and had a shorter latent period, an attempt was made to employ as young embryos as possible. When there was a shortage of embryos, use was occasionally made of the entire body, except the head, of quite small embryos. The tissue cultures were prepared from the lung tissue either immediately after its removal or on one of the two subsequent days, but no cultures were made from embryonic lung tissue more than three days old, owing to its poor growth-rate.

At the preparation of the tissue cultures, the lungs were minced into pieces of approximately 1 $mm^3$, or possibly even smaller, with uterine scissors in round-bottomed 40×100 mm test tubes. At the cutting, a sufficient quantity of nutrient medium was added to the minced lungs to prevent the pieces drying up during the mincing operation. To prevent

bacterial contamination, 100 units of penicillin and 100 μg of streptomycin per 1 ml had been added to the nutrient medium. After careful mincing, the tissue was washed three times with the nutrient medium.

*Storage of the Tissue.* — If pieces of minced tissue which had not been employed for tissue cultures on the same day, were used after one or two days storage in the refrigerator at +4°C, 20 ml of nutrient medium containing antibiotics were added to the tubes containing the minced tissue pieces and the tubes were then tightly closed with rubber stoppers.

*Preparation of the Nutrient Medium and Other Solutions Required. Amniotic Fluid.* Uteri of gravid cows were brought from the slaughterhouse within 2—3 hours of slaughtering. As a rule, uteri with fetuses 15—35 cm in length were selected. The amniotic fluid was removed from the uterus under sterile conditions. The surface of the uterus was cauterized with a free flame until it became discoloured and shrank, and the uterine wall was then punctured with the trocar (8 × 150 mm). No choice was made between the amniotic or allantoic sac, but the fluid obtained will be referred to as amniotic fluid in the following. The clear fluid escaping through the trocar was collected directly in sterile bottles of 200 ml capacity. One uterus usually yielded 1—1½ litres amniotic fluid. As an indicator of the amniotic pH, phenol red solution was added to each bottle to yield a final concentration of 0.002%. Amniotic fluids with a pH between 7.2 and 7.8 were accepted. The bottles were tightly closed with rubber stoppers and stored in an electrical deep freezer at —15° to —20°C. As a rule amniotic fluid more than one month old was not used for nutrient medium.

The amniotic fluid was immediately subjected to a sterility test in cooked liver broth tubes and also by preparing tissue cultures in media which had previously been proved to be sterile. In this latter case the growth of the tissue cultures was proof of sterility, and this test was found to be more reliable.

Immediately before its use as nutrient medium, 100 units of penicillin and 100 μg of dihydrostreptomycin per 1 ml were added to the amniotic fluid.

Owing to the short supply of amniotic fluid, it was used from the spring of 1954 onwards half and half with Parker's mixture 199, which was obtained ready-made from the pharmaceutical manufacturers Lääketehdas Orion Oy, Helsinki. It was stored in the refrigerator, and was used within a month of its preparation.

*Chicken Plasma* was prepared from heparinized blood taken from chickens by heart puncture. A hen usually yielded 50—70 ml and a cock 100 ml blood. The blood was centrifuged for 20 minutes at 2000 r.p.m. and the plasma obtained was divided in quantities of 1 ml into small test tubes, which were tightly closed with rubber stoppers and stored in the refrigerator at +4°C. Sterility tests were performed in the same way as with the amniotic fluid.

*Chick Embryo Extract* was prepared from embryonated chicken eggs incubated for 8—11 days after removal of the eyes. Equal amounts of chicken embryo and Parker's mixture 199 were minced for 1 minute in a Waring blendor. The suspension was incubated at 37°C for one hour and

then centrifuged for 20 minutes at 2000 r.p.m. The supernatant clear portion of the embryo extract was stored in small test tubes, 1 ml in each, in a deep-freezer at —15° to —20°C. Each preparation series was tested for sterility in the same manner as the amniotic fluid.

*Horse Serum.* Horse blood was collected at the slaughterhouse under sterile conditions and brought directly to the laboratory, where it was centrifuged for 20 minutes at 2000 r.p.m. The serum was separated and passed through a Seitz filter, after which it was stored in small test tubes in 2 ml lots, and inactivated at 56°C for ½ hour. The tubes were tightly closed with rubber stoppers and stored in a deep-freezer at —15° to —20°C. The serum was tested in the same manner as the amniotic fluid.

*Trypsin Inhibitor.* 1 g of trypsin inhibitor (Soy Bean Trypsin Inhibitor, G.B.I. Ohio) was dissolved in 200 g of Parker's mixture 199 and passed through a Seitz filter. It was divided into 1 ml lots placed in small test tubes which were tightly closed with rubber stoppers and stored in the refrigerator at +4°C. Its sterility was tested in the same way as that of the amniotic fluid, and Sabouraud's agar was used as an indicator of possible fungus infection.

*Phenol red* was prepared by dissolving 1 g of phenol red in 250 ml aq. dest., to which had been added 28.2 g 0.01 *N* NaOH. The solution was sterilized for 10 minutes at 120°C in the autoclave and kept in the refrigerator. 1 ml of this 0.4% storage solution was used per 200 g of amniotic fluid, resulting in an ultimate concentration of 0.002% phenol red in the nutrient medium.

*Heparin.* A solution of 0.5 mg of powdered heparin per ml was prepared in saline and sterilized by autoclaving for 10 minutes at 115°C. 0.5 ml of this heparin solution were used per 10 ml chicken blood.

The antibiotic content of the medium was: 100 units of penicillin and 100 $\mu$g of dihydrostreptomycin per 1 ml.

*Preparation of the Tissue Cultures Proper.* — Surgical asepsis was observed in the preparation of the tissue cultures.

One drop of chicken plasma, about ten tissue pieces and finally one drop of chick embryo extract were spread on the inner surface of the lower third of the test tube by a Pasteur pipette. As a result of the clotting of the chicken plasma and chick embryo extract the tissue pieces adhered to the wall of the test tube. The tissue culture tubes prepared in this manner were left to dry for ½—1 hour under a sterile cloth in an upright position in their frames. 1.5 ml of nutrient medium were then added. This nutrient medium consisted of equal amounts of amniotic fluid and Parker's mixture 199, 1% horse serum[1] and the following antibiotics: 100 units of

[1] In the investigations described in the literature (139), more horse serum has generally been used. In the present work, 5% of horse serum was used in the preliminary tests, but this seemed to inhibit in some degree the cytopathogenic effect of the poliomyelitis virus. On the other hand, when the horse serum was omitted entirely, non-specific cell degeneration began to appear in the tissue culture controls containing no viruses before the sixth day. Therefore 1% inactivated horse serum was used in the nutrient medium without any inhibitory effect upon the virus being observed.

penicillin and 100 μg of streptomycin per 1 ml. The tubes were tightly stoppered and were incubated in the rotary drums at 37°C. The tubes were tilted at 5° from the horizontal to prevent the nutrient medium from touching the stoppers. The outgrowth of the cultures in the tubes was observed daily in the microscope using a magnification of 80×. Usually after three days the outgrowth was satisfactory. In the presence of actively metabolizing tissue the pH fell and the red colour of the phenol red indicator turned yellow. Only cultures showing good cell growth were employed in the experiments.

*Maintenance of Tissue Cultures.* — In order to maintain the tissue cultures, the nutrient medium was changed every 3 or 4 days. The medium was renewed with the same quality and quantity of nutrient medium as before. The tissue cultures were then ready for use in the experiments. If they were not required immediately, they were stored in the incubator at +37°C, and the nutrient medium was changed again after 3 to 4 days.

## *The Method of Demonstrating the Growth of Viruses in Tissue Cultures*

The presence and reproduction of active viruses in the tissue cultures was demonstrated by means of the specific cytopathogenic effect of the viruses as indicated by cell degeneration (139).

Immediately upon change of the nutrient medium the tissue cultures were inoculated with 0.1 ml of the virus and the inoculated cultures were incubated at +37°C. After inoculation the cultures were examined microscopically (80 × magnification), initially every day, in order to follow the progress of the cell growth or degeneration. The degeneration increased with the time of influence of the virus. As a rule, the degree of cytopathogenicity was determined on the sixth day.

The degree of cytopathogenicity was denoted as follows:

— no fibroblastic degeneration,
\+ a few of the growing cells had degenerated,
++ less than half of the growing cells had degenerated,
+++ more than half of the growing cells had degenerated,
++++ all cells had degenerated.

Only the findings +++ and ++++ were considered positive, i.e., the virus had caused a cytopathogenic effect only if more than half of the cells had been destroyed by the sixth day.

As proof of the specificity of the cytopathogenic effect it was required that undegenerated growth should continue in the uninoculated control tubes on the sixth day and also that the homotypic antibody serum still inhibited the cytopathogenic effect of the viruses concerned on the sixth day.

*Assay of Viral Infectivity.* — The virus content in the culture fluids was determined by its infectivity to the outgrown cells in the tissue cul-

tures, the infectivity of the culture fluid being calculated according to the formula of Reed and Muench (135).

Serial 10-fold dilutions of the virus types to be tested were made and 0.1 ml of each dilution was introduced into each of a set of five tubes, which were incubated for six days at 37°C. The endpoint of viral infectivity was expressed as $TCD_{50}$, which equals 50% of tissue cultures degenerated with this dilution of the virus type investigated. The titre of the virus was expressed in terms of the 10-fold dilution at which 1 ml contained one $TCD_{50}$. All titres have been given as initial dilutions per 1 ml.

According to the suggestion of the National Foundation for Infantile Paralysis, at the inoculation of the viruses the dilution used in the neutralization tests was such that 0.1 ml contained 100 $TCD_{50}$ (25).

In numerous measurements performed after different passages this $TCD_{50}$ varied to some extent, although not more than within $10^{-1}$, usually within $10^{-0.5}$. Since the titre of type I was on an average $10^{-5.5}$—$10^{-6}$, that of type II $10^{-4.5}$—$10^{-5}$ and that of type III $10^{-5.5}$—$10^{-6}$, the dilutions $10^{-3}$, $10^{-2}$ and $10^{-3}$, from which 0.1 ml was taken, were as a rule used in the neutralization tests for types I, II and III, respectively.

*Storage of Viruses and Decrease in Titre.* — The virus strains in small quantities intended to be used at one time were stored in an electrical deep-freezer at —15° to —20°C in amniotic solution.

If at titration in the neutralization test series the virus titre was observed to have fallen, the viruses were cultivated again by one passage in the tissue cultures. This was done on an average every two months. The titre of type II displayed a greater tendency to decrease than that of the others.

After each tissue culture passage the virus was retitrated.

*Multiplication of Viruses in Culture.* — In virus cultivation it is necessary to know when the titre is at its highest. Robbins, Weller and Enders found that in tissue cultures prepared from human embryonic tissue the titre of the Lansing virus attained its maximum height during the fourth to eighth days, sometimes not even before the tenth day after inoculation and that it made hardly any difference whether the titre of the virus in the 0.1 ml inoculum was $10^{-1}$ or $10^{-3}$. In the latter case the titre was slightly higher than in the former, except for the first few days (139).

THE MULTIPLICATION OF BRUNHILDE VIRUS
(100 $TCD_{50}$ of $10^{-6}$/0.1 ml)

| Days after inoculation | Virus titre [1] |
|---|---|
| 3 | 5.7 |
| 4 | 5.6 |
| 5 | 6.1 |
| 6 | 6.0 |
| 7 | 5.8 |
| 8 | 5.9 |
| 9 | — |
| 10 | 5.4 |

[1] In negative powers.

Similar experiments were carried out in our laboratory. For instance, in our tissue cultures the inoculum 0.1 ml, with 100 $TCD_{50}$ of Brunhilde strain which had the titre $10^{-6}$, gave the titres shown in the table above determined on successive days and calculated according to Reed and Muench. The sixth day was chosen as harvesting day. The same procedure was followed for the other test virus strains and gave similar results.

### *Neutralization Test Procedure*

During renewal of the nutrient medium trypsin inhibitor was added to the medium in a concentration of 0.025 mg per 1 ml. The lysis of the plasma clotting was prevented by this substance (107).

For neutralization, equal volumes (0.3 ml) of undiluted, inactivated serum and of the separate virus dilutions in a concentration of 100 $TCD_{50}$ per 0.1 ml were mixed well together and allowed to stand at room temperature for one hour.

In order to indicate the neutralization, 0.2 ml of the serum-virus mixture were inoculated from each neutralization tube into a duplicate set of tissue culture tubes. Each tissue culture tube then contained 0.1 ml of the serum and 100 $TCD_{50}$ in 0.1 ml of the virus. Each serum was thus introduced into six tissue cultures, two for each virus type. The inoculated tissue cultures were incubated at + 37°C in the rotary drum. They were examined on the fourth and sixth days after inoculation. Since the virus control cultures had not always degenerated completely on the fourth day, in fact in some cases not before the sixth day, it was frequently necessary to consider the sixth day as the test period for proof of cytopathogenicity (v. p. 52). The cultures were not observed at longer intervals, in order to avoid the virus »break-through» (83). As in the virus infectivity determinations, the results of the neutralization test were denoted by the symbols —, +, ++, +++ and ++++ (p. 48). Only the findings +++ and ++++ were considered proof of the cytopathogenic effect of the virus, i.e., proof of the fact that the serum under investigation was incapable of neutralizing the virus.

#### *Controls*

*Virus Controls.* — In the initial stage of the work, a virus control investigation in duplicate sets was carried out on the effect of the virus dilution which was used for the neutralization test. They were examined microscopically on the fourth and sixth days. The results were accepted, if at least partial degeneration of the cells (+++) could be observed by the fourth day and complete degeneration (++++) on the sixth day. The major part of the work was controlled by titrating each virus type whenever the neutralization test was employed. The titrations were performed in 10-fold dilutions and they were extended so far that the cells of the tissue cultures grew without any degeneration whatsoever in the last dilution. The titre, calculated according to the method of Reed and

Muench, showed that the virus quantity had frequently been somewhat less than 100 $TCD_{50}$.

*Tissue Culture Controls.* — As tissue culture controls, six cultures were left uninoculated in each neutralization test series. For the results of the neutralization test to be accepted, these cultures had to show good growth without cell degeneration on the sixth day after the test.

*Virus Type Controls.* — Approximately half-way through the present work tests of the virus types with known type-specific sera [1] were commenced. At first these tests were performed only occasionally but towards the end of the work they were effected in connection with every neutralization test. Duplicate neutralization tests with the known type-specific serum were made of each virus type in the standard test dilution. The results were accepted, if these tissue cultures showed good growth without cell degeneration on the sixth day.

*Serum Controls.* — The potential cytopathogenic effect of the sera under investigation was not investigated as a routine, except in those cases in which cytopathogenicity occurred with every type.

### *Criticism of the Neutralization Test*

In the neutralization tests performed on tissue cultures many variables are involved; consequently there are many chances of error.

The *tissue* can affect the results in one of several ways:

*Material from Different Embryos.* — As an embryo is fit for use at most for three days, almost each neutralization test series has to be carried out with tissue from different embryos, which may differ in their susceptibility to the poliomyelitis virus.

A comparative investigation (83) with tissue cultures from monkeys' testes showed that the titre of one and the same virus dilution varied only within $10^{-0.5}$ in tissue cultures prepared from the testes of different monkeys.

A question of particular importance is whether the tissue pieces derived from the embryo of an immune mother contain poliomyelitis antibodies which might neutralize the poliomyelitis viruses and thus cause different results to be obtained in the neutralization test than would be obtained with tissue cultures from the embryo of a susceptible mother. The antibodies circulating in the blood were eliminated by washing the tissue pieces of embryonic lungs before the cultures were prepared, so that it is the tissue immunity of the pieces which is significant in this respect. In a study of this question (82) with unwashed tissue cultures from monkeys' testes it was found that the titre of the poliomyelitis virus was identical in the case of immune and non-immune monkeys.

The number of cells growing in the tissue cultures may have an influence upon the growth of the virus. An investigation (181) with trypsinated cell cultures of monkey's kidney showed that the titre of the viruses

[1] Type-specific poliomyelitis antisera from hyper-immunized monkeys were kindly supplied to the laboratory by Dr. J. L. Melnick.

is higher, the greater the number of growing cells in the tissue cultures. Yet, according to other investigators the titre of the virus remained unchanged in spite of great variations in the number of cells in the tissue cultures (139), even when the number of cells was increased as much as tenfold (84). An attempt has been made in the present investigation to use approximately ten pieces of minced tissue for each tissue culture.

The age of the embryo may have some influence, inasmuch as the tissues of a younger embryo grow more rapidly and, consequently, the number of growing cells is greater than in the case of an older embryo.

The age of the tissue pieces taken from the embryo affects the quality of the cells. In the opinion of the author the cells in tissue cultures prepared from a newly operated embryo have thinner walls and more uniform size than tissue cultures prepared as late as on the third day, where the cells have thicker walls and are not as suspectible to the effect of the virus as the former.

The *amniotic fluid* is different in the various neutralization tests. The amniotic fluid from different cows may differ in its effect on the growth of the virus since the serum of cows, too, has been found to contain varying quantities of antibodies to poliomyelitis (53).

*Different horse sera* may have a different inhibitory effect upon the growth of the poliomyelitis virus. Robbins, Weller and Enders (139) have shown experimentally that horse serum has an inhibitory effect upon the growth of the Lansing virus, if 20% of the serum is added to the nutrient medium, but none when only 5% is added. In order to prevent the non-specific degeneration of the tissue cultures, these authors suggested the use of 5% of horse serum. In the writer's experience an addition of 5% occasionally inhibited the growth of the viruses; for this reason in the present investigation only 1% of horse serum was added to the nutrient medium to prevent non-specific cell degeneration (v. p. 47). Hammon (53) found varying quantities of antibodies in the sera of horses.

The *amount of virus in the inoculum* may show slight variations, owing to technical reasons and to the fact that the viruses are derived from different passages, resulting in a slightly varying titre. The dilution of the virus to the dosage value was usually done with an accuracy of $10^{-0.5}$. When each virus type was titrated in connection with the different neutralization test series, it was generally found that the virus quantity had been somewhat below 100 $TCD_{50}$. The greatest difficulties were encountered with type II, the titre of which showed the strongest tendency to decrease.

As has been stated before, the titre of type I was usually $10^{-5.5}$—$10^{-6}$, that of type II $10^{-4.5}$—$10^{-5}$ and that of type III $10^{-5.5}$—$10^{-6}$; consequently, $10^{-4}$, $10^{-3}$ and $10^{-4}$ was used as dosage dilutions for type I, II and III, respectively. All dilutions have been stated as initial dilutions per 1 ml.

The *examination time for the neutralization test* is of significance for the results (83). The virus control cultures determined whether the test period should be four or six days. If the virus control cultures had completely degenerated by the fourth day, the neutralization tests were »read» on

that day. If complete degeneration had not yet taken place, the experimental results were taken on the sixth day; this was necessary in quite a number of cases. Thus, complete degeneration of the virus controls was required before the results were accepted.

The *potential gradual change of the procedure* during the course of the work. The practical work in the present investigation took 20 months (January, 1954—August, 1955), and it is thus possible that unintentional, small changes took place in the procedure. For the first 8—9 months the antibodies of the inhabitants of Helsinki were studied exclusively. After this time the blood samples obtained from the rural districts were investigated on the basis of their consecutive numbers, without any knowledge of locality and of the age of the person concerned. Repeated determinations, including also persons living in Helsinki, were made toward the end of this work at the same time as the samples collected in January 1955 in Central Finland were dealt with.

The same person has taken the results throughout the investigation, the author having personally inspected all tissue cultures and tests under the microscope.

### *Replications*

Since an experimental procedure such as the one used in this investigation always contains many other potential technical sources of error apart

TABLE 10

REPLICATIONS

| Type of virus | First determination | No of replications | Second determination | | | Deviation % |
|---|---|---|---|---|---|---|
| | | | — | ± | + | |
| I | — | 204 | 185 | 7 | [11] | 5.4 |
| | ± | (47) | 21 | 5 | 21 | — |
| | + | 360 | [6] | 7 | 347 | 1.7 |
| II | — | 241 | 214 | 11 | [16] | 6.7 |
| | ± | (51) | 23 | 7 | 21 | — |
| | + | 365 | [32] | 2 | 331 | 8.8 |
| III | — | 193 | 179 | 3 | [11] | 5.7 |
| | ± | (58) | 36 | 3 | 19 | — |
| | + | 345 | [16] | 5 | 324 | 4.6 |
| I, II and III | +or— | 1708 | all deviations 92 | | | 5.4 |
| | ± | (156) | | | | |
| | Total | 1864 | | | | |

from those discussed above, many determinations were repeated. Table 10 shows the changes in the results produced by these repeated determinations.

Determinations giving the same result as the first determination in one of the duplicate tests and the opposite result in the other test have not been considered as contradicting the previous finding, since such a serum obviously has a low titre, and only a series of many replication tests would decisively place this serum in one or the other category.

A study of the results will show that greater deviations were obtained in the neutralizing test for the type II virus. The titre of this virus type, in fact, was the most labile. With regard to the more stable titre of other types, particularly type I but also type III, more deviations were found in the tests where no antibodies were established, i.e., in cases where the cells had degenerated in the tissue culture. This may be due either to unspecific degeneration in the first test or to a low titre at which the serum antibodies cannot always become apparent.

In all 5.4% of the determinations were found to differ from the previous findings. This probably represents a high estimate since the cases chosen for repeated determinations were mainly those in which the first results had appeared more or less uncertain.

## RESULTS

### I CITY OF HELSINKI

In Helsinki, the poliomyelitis neutralizing antibodies were determined in 1084 persons. The collection of the sera is explained on page 43. Their age and sex distribution is given in Table 11.

#### *The Incidence of Poliomyelitis Neutralizing Antibodies*

Table 11 shows the incidence by age and sex of neutralizing antibodies to poliomyelitis virus type I in the inhabitants of Helsinki before the 1954 poliomyelitis epidemic.

The notation $\pm$ (partially positive reaction) has been used in the table to distinguish those neutralization tests in which only one of the duplicate tests showed the presence of antibodies. These sera have been accepted as containing antibody with a low titre. Similar cases occur throughout the series in approximately the same low proportions as in this table. In the subsequent tables, they are included in the sera with antibodies and are not distinguished.

Table 11 shows that the percentual occurrence of antibodies is similar in males and females.

TABLE 11

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO TYPE I POLIOMYELITIS VIRUS IN HELSINKI

| Age in years | Number examined | | | Sera with antibodies against type I | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number + | | | | | Per cent + | | | Standard error |
| | Male | Female | Total | Male | | Female | | Total | Male | Female | Total | |
| | | | | + | ±[1] | + | ±[1] | + | | | | |
| 0— 4 | 51 | 32 | 83 | 15 | (1) | 8 | — | 23 | 30 | 25 | 28 | 4.9 |
| 5— 9 | 90 | 77 | 167 | 50 | (1) | 48 | — | 98 | 56 | 62 | 59 | 3.8 |
| 10—14 | 68 | 52 | 120 | 48 | (1) | 36 | — | 84 | 70 | 69 | 70 | 4.2 |
| 15—19 | 19 | 48 | 67 | 13 | (1) | 32 | (1) | 45 | 68 | 67 | 67 | 5.7 |
| 20—29 | 51 | 104 | 155 | 35 | (1) | 69 | — | 104 | 69 | 66 | 67 | 3.8 |
| 30—39 | 63 | 102 | 165 | 48 | — | 83 | (1) | 131 | 76 | 81 | 79 | 3.2 |
| 40—49 | 40 | 116 | 156 | 33 | — | 104 | — | 137 | 82 | 90 | 88 | 2.6 |
| ≥50 | 47 | 124 | 171 | 43 | — | 120 | — | 163 | 91 | 97 | 95 | 1.7 |
| Total | 429 | 655 | 1084 | 285 | (7) | 500 | (2) | 785 | • • | • • | • • | • • |

[1] one of duplicate tests: +, the other: —.

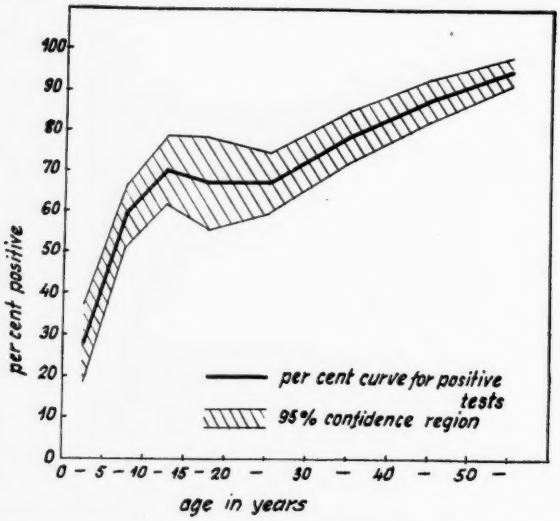


Fig. 7. — Age specific frequency of neutralizing antibodies to type I poliomyelitis virus in Helsinki in 1954 before the outbreak of the poliomyelitis epidemic.

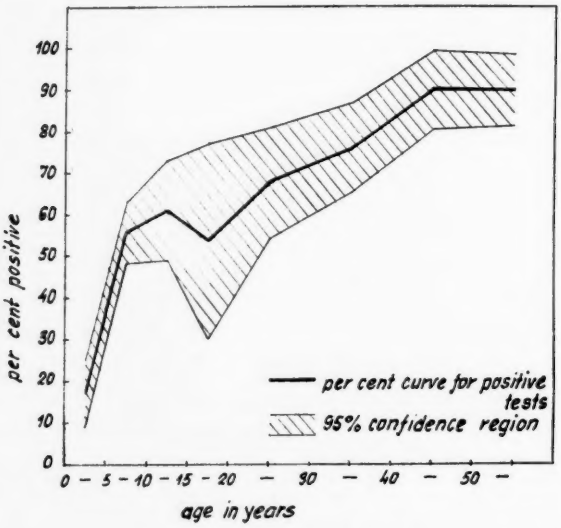


Fig. 8. — Age specific frequency of neutralizing antibodies to type II poliomyelitis virus in Helsinki in 1954 before the outbreak of the poliomyelitis epidemic.

Figure 7 gives the neutralizing antibody rates for males and females together. As in the table, the results have been given in 5-year groups for persons under 20 years of age and in 10-year groups for persons over 20. The value for each group has been plotted against the centre of the age interval. The 95% confidence limits have been entered in the figure for each 5- and 10-year group. The width of the 95% confidence interval in both directions from the observed percentage equals twice the standard error.

The results reveal that neutralizing antibodies to poliomyelitis virus type I were present in 28% of the children under 5 years. When the series of 83 sera investigated is divided into one-year age groups, as has been done in the table, the one-year groups are found to be too small to allow any inferences to be drawn. However, it appears likely that the percentage of children with neutralizing antibodies to type I increases most sharply between the ages of 4 and 5. The 50% limit is attained in 5-year-old children. 59% of children between 5 and 9 years revealed neutralizing antibodies to type I. The figure rises to 70% in persons between 10 and 15 years and maintains this level through the 20—29 year group. In persons between 40 and 50 these antibodies occurred in nearly 90%, in persons over 50 in 95% of the cases investigated.

The incidence of poliomyelitis neutralizing antibodies to types II and III in the Helsinki group is shown in Table 12. Here, too, the results for males and females are given separately; the percentual occurrence in the different age groups is clearly similar in both sexes. Figs. 8 and 9 show the percentual occurrence by age groups for types II and III, respectively.

Fig. 8 shows the percentage of persons with neutralizing antibodies to type II in the different age groups. 17% of the children under 5 years possessed antibody of this kind, and it will be seen that the antibody-rate increased sharply between the ages of 4 and 5 years, the 50% limit being reached in 5-year-old children. 56% of the children between 5 and 9 years, 61% of the children between 10 and 14 and only 54% of the persons between 15 and 19 had neutralizing antibodies to type II. This fall in the antibody rate in the higher age group may be real, but it may also be attributable to a random error due to the small size (67 persons) of the group. In the 20—29-year group antibodies occurred in 68%, of the cases,

## TABLE 12

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO TYPES II AND III POLIOMYELITIS VIRUS IN HELSINKI

| Age in years | Number examined | | | Sera with antibodies against | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Type II | | | | | Type III | | | |
| | | | | Number + | | | % | Standard error | Number + | | | % | Standard error |
| | Male | Female | Total | Male | Female | Total | + | | Male | Female | Total | + | |
| 0— 4 | 51 | 32 | 83 | 8 | 5 | 13 | 17 | 4.1 | 17 | 8 | 25 | 30 | 5.0 |
| 5— 9 | 90 | 77 | 167 | 49 | 45 | 94 | 56 | 3.8 | 52 | 49 | 101 | 60 | 3.8 |
| 10—14 | 68 | 52 | 120 | 43 | 30 | 73 | 61 | 6.0 | 45 | 40 | 85 | 71 | 5.5 |
| 15—19 | 19 | 48 | 67 | 8 | 28 | 36 | 54 | 11.7 | 7 | 30 | 37 | 55 | 11.7 |
| 20—29 | 51 | 104 | 155 | 36 | 70 | 106 | 68 | 6.6 | 33 | 74 | 107 | 69 | 6.5 |
| 30—39 | 63 | 102 | 165 | 51 | 75 | 126 | 76 | 5.4 | 49 | 77 | 126 | 76 | 6.0 |
| 40—49 | 40 | 116 | 156 | 36 | 105 | 141 | 90 | 4.8 | 36 | 105 | 141 | 90 | 4.8 |
| ≥50 | 47 | 124 | 171 | 39 | 114 | 153 | 90 | 4.4 | 43 | 111 | 154 | 90 | 4.4 |
| Total | 429 | 655 | 1084 | 270 | 472 | 742 | . . | . . | 282 | 494 | 776 | . . | . . |

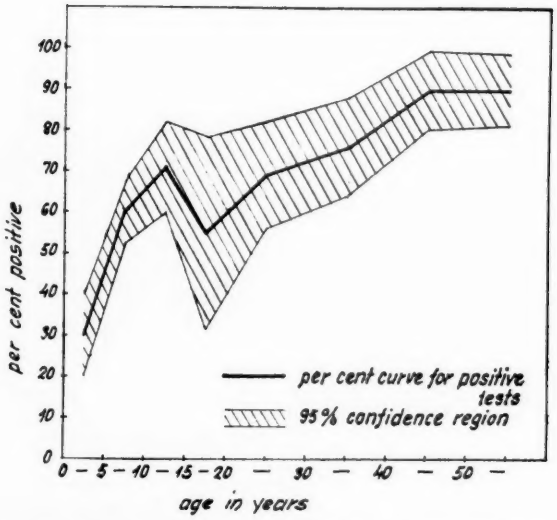


Fig. 9. — Age specific frequency of neutralizing antibodies to type III poliomyelitis virus in Helsinki in 1954 before the outbreak of the poliomyelitis epidemic.

after which the rate rose gradually with increasing age, finally reaching 90% in the persons over 50.

Fig. 9 shows the percentual incidence of neutralizing antibodies to type III in the different age groups. Thus, 30% of the children under 5 years showed antibodies, once again with a strong increase in the rate between the ages of 4 and 5. The 50% limit was reached in the five-year-old children. Children between 5 and 9 years had neutralizing antibodies to type III in 60%, children between 10 and 14 in 71% and persons between 15 and 19 in 55% of the cases. A decrease, similar to that found with type II virus, can, thus, be observed in the type III antibody rate of this group. This decrease cannot be considered statistically significant, as will be seen from the 95% confidence limits shown in the figure. The percentage of persons with antibodies to type III increased slowly with age, reaching 90% in persons between 40 and 49 years of age and 95% in the persons over 50.

The curves indicating the percentual distribution by age of the poliomyelitis neutralizing antibodies to the different virus types in the sera of persons from Helsinki bear a close resemblance

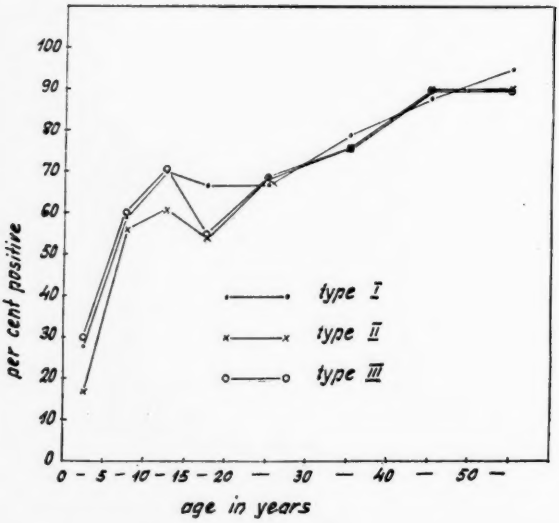


Fig. 10. — Age specific frequencies of neutralizing antibodies to the three types of poliomyelitis virus in Helsinki in 1954.

to each other. In Fig. 10 the curves showing the poliomyelitis neutralizing antibody incidence for the different virus types have been plotted together.

### *The Incidence of Combinations of Poliomyelitis Neutralizing Antibodies*

Table 13 gives by age group detailed results of the simultaneous occurrence in one person of different neutralizing antibodies, both in absolute numbers and in percentages.

Fig. 11 indicating the same results contains three curves, the first indicating sera with neutralizing antibodies to three types, the second showing the total of sera with antibodies to three or two types, and the third the total number of sera with antibodies to three, two or one types. As in previous figures persons under 20 years of age have been divided into 5-year groups and persons over 20 into 10-year groups; the percentages for each group have been plotted against the centre of the age interval.

A study of this figure reveals that, on an average, the curve for sera with three neutralizing antibodies rises with increasing age.

## TABLE 13

OCCURRENCE OF DIFFERENT COMBINATIONS OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN HELSINKI 1954

Number

| Age group | Number | Three antibodies | Two antibodies | | | | One antibody | | | | No antibody |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | I+II | I+III | II+III | Total | I | II | III | Total | |
| 0— 4 | 83 | 6 | 10 | — | 15 | 25 | 7 | 2 | — | 9 | 43 |
| 5— 9 | 167 | 59 | 5 | 6 | 23 | 34 | 25 | 12 | 4 | 41 | 33 |
| 10—14 | 120 | 50 | 9 | 6 | 13 | 28 | 11 | 15 | 6 | 32 | 10 |
| 15—19 | 67 | 23 | 5 | 9 | 13 | 27 | 2 | 7 | 2 | 11 | 6 |
| 20—29 | 155 | 65 | 9 | 4 | 20 | 33 | 26 | 7 | 11 | 44 | 13 |
| 30—39 | 165 | 100 | 2 | 3 | 16 | 21 | 16 | 8 | 7 | 31 | 13 |
| 40—49 | 156 | 116 | — | 1 | 4 | 5 | 16 | 9 | 8 | 33 | 2 |
| ≧50 | 171 | 141 | 1 | 2 | 8 | 11 | 4 | 8 | 6 | 18 | 1 |
| Total | 1084 | 560 | 41 | 31 | 112 | 184 | 107 | 68 | 44 | 219 | 121 |

Per cent

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0— 4 | 100 | 7 | 12 | — | 18 | 30 | 8 | 2 | — | 11 | 52 |
| 5— 9 | 100 | 35 | 3 | 4 | 14 | 20 | 15 | 7 | 2 | 25 | 20 |
| 10—14 | 100 | 42 | 8 | 5 | 11 | 23 | 9 | 12 | 5 | 27 | 8 |
| 15—19 | 100 | 34 | 8 | 13 | 19 | 40 | 3 | 10 | 3 | 16 | 9 |
| 20—29 | 100 | 42 | 6 | 3 | 13 | 21 | 17 | 4 | 7 | 28 | 8 |
| 30—39 | 100 | 61 | 1 | 2 | 10 | 13 | 10 | 5 | 4 | 19 | 8 |
| 40—49 | 100 | 74 | — | 1 | 2 | 3 | 10 | 6 | 5 | 21 | 1 |
| ≧50 | 100 | 82 | 1 | 1 | 5 | 6 | 2 | 5 | 3 | 11 | 1 |

Similarly the curve representing the total number of sera with either three or two antibodies follows approximately the same course as the antibody curves for the individual virus types (Figs. 7, 8 and 9).

The most important group comprises the persons without neutralizing antibodies. Their number is seen from Table 13 to amount to 52% in the case of children under 5 years, 20% among children between 5 and 9 years, and 8—9% among persons between 10 and 40 years. Of the persons over 40 years only 1% belong to this class.

Table 13 further reveals that among sera showing neutralizing antibodies to two virus types the combination of type II and type III antibody is most common (61%). The combination of types I and II occurs in 22% and the combination I + III in 17% of all such cases.

Among the sera with antibodies to one type only, 49% contain antibodies to type I, 31% to type II and 20% to type III.

### *The Incidence of Poliomyelitis Neutralizing Antibodies in Different Social Classes in Helsinki*

The material from Helsinki has been divided into four social classes according to the classification, based on profession, used by the Statistics Office of the City of Helsinki. This method was

TABLE 14

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE DIFFERENT SOCIAL CLASSES IN HELSINKI

| Age groups | I social class (highest incomes) | | | | II social class | | | | III social class | | | | IV social class | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No of tested | % positive | | | No of tested | % positive | | | No of tested | % positive | | | No of tested | % positive | | |
| | | I | II | III | | I | II | III | | I | II | III | | I | II | III |
| 0— 4 | 14 | 15 | 15 | 23 | 18 | 22 | 11 | 33 | 35 | 37 | 17 | 34 | 16 | 25 | 19 | 25 |
| 5— 9 | 37 | 54 | 57 | 68 | 44 | 57 | 55 | 52 | 59 | 58 | 58 | 64 | 24 | 75 | 54 | 54 |
| 10—19 | 45 | 73 | 56 | 62 | 26 | 73 | 73 | 73 | 35 | 74 | 69 | 69 | 68 | 62 | 57 | 62 |
| 20—29 | 17 | 65 | 65 | 59 | 26 | 77 | 65 | 69 | 76 | 67 | 71 | 71 | 21 | 57 | 81 | 81 |
| 30—39 | 32 | 78 | 69 | 66 | 53 | 81 | 87 | 83 | 43 | 84 | 74 | 79 | 23 | 83 | 74 | 87 |
| 40—49 | 19 | 74 | 95 | 84 | 43 | 88 | 93 | 93 | 50 | 92 | 86 | 90 | 29 | 86 | 97 | 97 |
| ≥50 | 24 | 97 | 97 | 100 | 43 | 93 | 86 | 91 | 46 | 98 | 85 | 85 | 42 | 98 | 95 | 95 |
| Total | 188 | .. | .. | .. | 253 | .. | .. | .. | 344 | .. | .. | .. | 223 | .. | .. | .. |

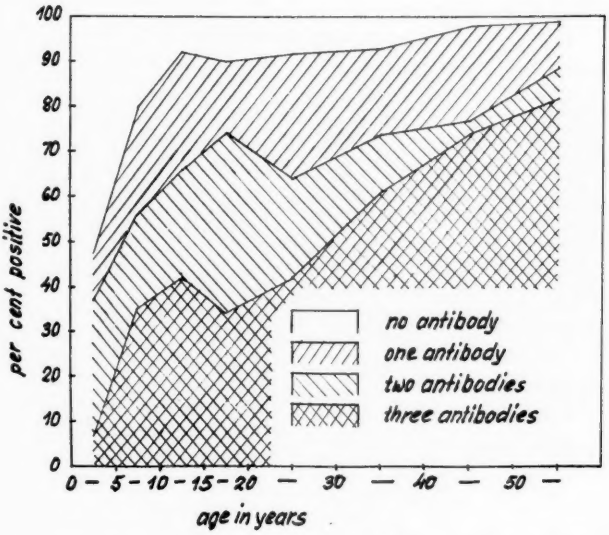


Fig. 11. — Age specific frequency of combinations of the neutralizing antibodies to the three types of poliomyelitis virus in Helsinki in 1954.

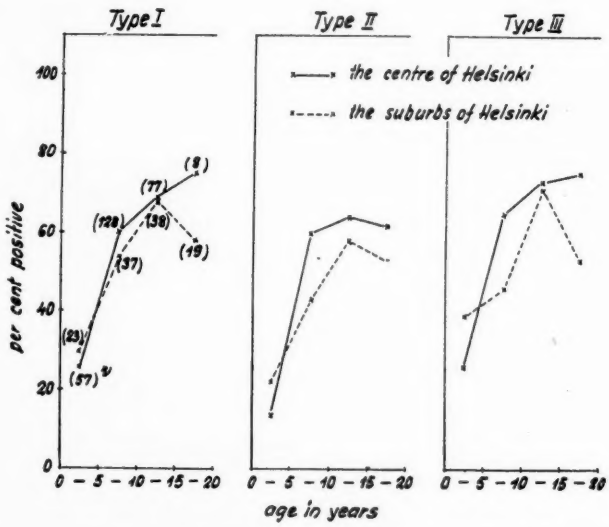


Fig. 12. — Comparison of age specific frequencies of neutralizing antibodies to the three types of poliomyelitis virus in the centre and in the suburbs of Helsinki.

* Number tested in each age group in brackets.

used earlier in classifying the clinical poliomyelitis cases (p. 40). The author regards the division by profession as vague and unsatisfactory, but it was the only classification method available for use in the present material.

Table 14 gives the percentages for those cases in which the profession of the person could be ascertained. No systematic differences whatsoever can be observed in the incidence of antibodies in these groups.

### *The Incidence of Poliomyelitis Neutralizing Antibodies in the Centre and in the Suburbs of Helsinki*

The higher, clinical poliomyelitis morbidity in the suburbs of Helsinki than in the city centre during the 1954 epidemic (130) prompted the question whether the neutralizing antibodies to poliomyelitis virus might show a different occurrence in these areas. For this reason, in those persons under 20 whose domicile could be ascertained, the percentual occurrence by age was determined separately for the centre and the suburbs of Helsinki.

These percentages have been plotted according to age in Fig. 12. From these curves, on which the absolute numbers are given in brackets, it is evident that no distinct, statistical difference exists between the two areas.

## II RURAL AREAS

### *IIa The Kuhmoinen District*

The location of the rural district of Kuhmoinen can be seen from the map in Fig. 13.

The Kuhmoinen district, including for the purposes of the present investigation the rural communes of Kuhmoinen, Padasjoki and Luopioinen, was selected because poliomyelitis has occurred there comparatively rarely, according to the reports on infectious diseases made to the State Medical Board.

The Kuhmoinen district has a fairly isolated position on a water-shed, surrounded by lakes discharging partly westward into the Kokemäenjoki system and partly eastward into the Kyminjoki system. The area has no railway, being connected with its surroundings only by bus services. The aggregate area of the

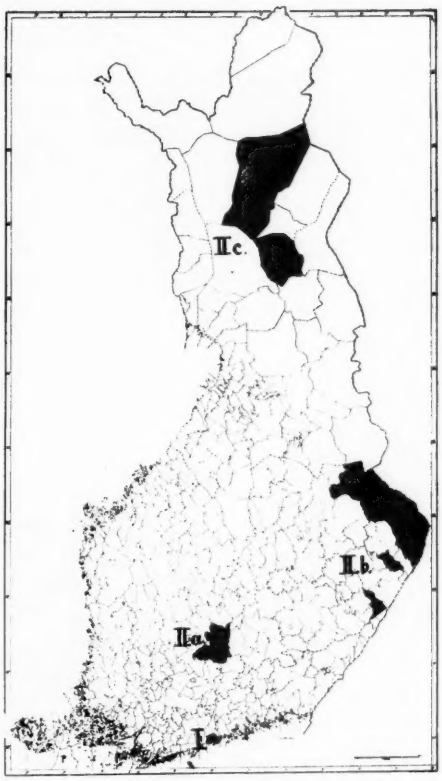


Fig. 13.

The investigated districts: I The city of Helsinki
II The rural districts
a) The Kuhmoinen district
b) The Joensuu district
c) The Kemijärvi district

district is 1478 km² and in 1954 the number of inhabitants was, 17,111, giving a population density of 11.6 per km².

*Previous Poliomyelitis Cases:* During the period 1939—53 a total of 9 poliomyelitis cases were reported from this area, 2 cases in 1940, 1 in 1943, 2 in 1946, 3 in 1947 and 1 in 1950. The mean annual morbidity per 100,000 members of the population is thus 3.5 for this period. Unfortunately, the reports contain no information on the age distribution of the poliomyelitis cases.

The sera of 552 persons from this area were investigated for neutralizing antibodies to the three poliovirus types. 248 serum

samples, collected in the summer of 1954, were investigated first, but, when they gave different results from the Helsinki samples, they were complemented in January 1955 by further blood samples, collected by proportional sampling. 304 of this second batch of sera were investigated for neutralizing antibodies to the three virus types.

Between the two sampling periods, in the summer of 1954, two poliomyelitis cases were reported from this area: a 9-year-old boy with nonparalytic poliomyelitis and a 30-year-old woman who contracted paralytic poliomyelitis with respiratory paralysis. Poliomyelitis virus type I was isolated from the faeces of the boy (111). He had been infected by his brother who was doing his military service in another locality, where he had contracted paralytic poliomyelitis.

### *The Incidence of Poliomyelitis Neutralizing Antibodies*

The incidence of neutralizing antibodies to the three types of poliomyelitis virus in the different age groups in the entire material from Kuhmoinen is presented in Table 15 and Fig. 14. Since no difference in the occurrence of antibodies was observed between males and females, both sexes have been treated together.

The curves have been plotted according to the same method as that used for the Helsinki results, but no confidence limits have been shown in the figure. The standard error of percentage for each age group has been given in the table. The 95% confidence limit equals twice the standard error.

A study of the figure reveals that neutralizing antibodies to type I occurred in this material in 11% of the children under 5 years, in 34% of the children between 5 and 9, and then maintained roughly the same level up to 15 years of age, where they surpassed the 50% limit. The occurrence of antibodies in persons between 15 and 19 years was 58%, increasing gradually to 81% in persons over 40.

Neutralizing antibodies to type II occurred in 2% of the children under 5 years, in 25% of the children between 5 and 9, in 41% of those between 10 and 14, and reached the 50% limit in children at 15 years of age. The subsequent age groups showed a continuous increase up to 86% in persons over 40.

TABLE 15

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE KUHMOINEN DISTRICT

| Age in years | Number examined | Sera with antibodies against | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Type I | | | Type II | | | Type III | | |
| | | Number + | % + | Standard error | Number + | % + | Standard error | Number + | % + | Standard error |
| <1 | — | — | | | — | | | — | | |
| 1 | 1 | — | | | — | | | — | | |
| 0—4 2 | 47 6 | 5 — | 11 | 4.6 | 1 — | 2 | 2.0 | 7 2 | 15 | 5.2 |
| 3 | 15 | 1 | | | — | | | 3 | | |
| 4 | 25 | 4 | | | 1 | | | 2 | | |
| 5 | 16 | 2 | | | 1 | | | 2 | | |
| 6 | 22 | 9 | | | 6 | | | 2 | | |
| 5—9 7 | 96 16 | 33 6 | 34 | 4.9 | 24 3 | 25 | 4.4 | 27 6 | 28 | 4.6 |
| 8 | 22 | 9 | | | 9 | | | 9 | | |
| 9 | 20 | 7 | | | 5 | | | 8 | | |
| 10—14 | 98 | 36 | 37 | 4.9 | 40 | 41 | 5.0 | 48 | 49 | 5.1 |
| 15—19 | 91 | 53 | 58 | 5.2 | 52 | 57 | 5.2 | 51 | 56 | 5.2 |
| 20—29 | 82 | 60 | 73 | 4.9 | 56 | 68 | 5.2 | 49 | 60 | 5.4 |
| 30—39 | 74 | 47 | 64 | 5.6 | 55 | 74 | 5.1 | 49 | 66 | 5.5 |
| ≥40 | 64 | 52 | 81 | 4.9 | 55 | 86 | 4.4 | 56 | 88 | 4.1 |
| Total | 552 | 286 | .. | .. | 283 | .. | .. | 287 | .. | .. |

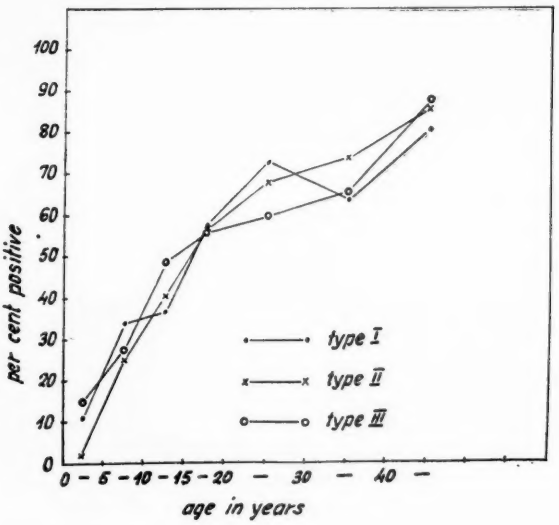


Fig. 14. — Age specific frequencies of neutralizing antibodies to the three types of poliomyelitis virus in the Kuhmoinen district in 1954.

Neutralizing antibodies to type III occurred in 15% of the children under 5 years, in 28% of the children between 5 and 9 and in 49% of those between 10 and 15, during which period the 50% limit was reached. The antibody rate then rises slowly, the percentage amounting to 66% in persons between 30 and 39, compared with 88% for persons over 40.

The curves for the different types show a remarkable similarity. The same observation was made in the curves from the Helsinki material.

Tables 16 and 17 give the separate results for the blood samples collected in June 1954 and for those obtained in January 1955, both as absolute figures and percentages. Figs. 15, 16 and 17 give the results from the two series for each virus type. The 95% confidence limits for the results of the June, 1954 series have also been shown in each figure. The figures show that the results of the January, 1955 series fall within the confidence interval of the results of the June, 1954 series. Thus, no statistically significant

TABLE 16

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE KUHMOINEN DISTRICT IN JUNE 1954

| Age in years | Number examined | Sera with antibodies against | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Type I | | | Type II | | | Type III | | |
| | | Number + | % + | Standard error | Number + | % + | Standard error | Number + | % + | Standard error |
| 0— 4 | 31 | 3 | 10 | 5.5 | 1 | 3 | 3.1 | 5 | 16 | 6.7 |
| 5— 9 | 46 | 17 | 37 | 7.2 | 9 | 20 | 6.0 | 14 | 29 | 6.7 |
| 10—14 | 32 | 15 | 47 | 9.0 | 15 | 47 | 8.9 | 17 | 53 | 9.0 |
| 15—19 | 35 | 20 | 57 | 8.5 | 22 | 63 | 8.3 | 19 | 54 | 8.5 |
| 20—29 | 35 | 25 | 71 | 7.8 | 23 | 66 | 8.1 | 21 | 60 | 8.4 |
| 30—39 | 36 | 25 | 69 | 7.8 | 28 | 78 | 7.0 | 24 | 67 | 7.9 |
| ≥40 | 33 | 28 | 85 | 6.3 | 31 | 94 | 4.2 | 31 | 94 | 4.2 |
| Total | 248 | 133 | .. | .. | 129 | .. | .. | 131 | .. | .. |

TABLE 17

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE KUHMOINEN DISTRICT IN JANUARY 1955

| Age in years | Number examined | Sera with antibodies against | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Type I | | | Type II | | | Type III | | |
| | | Number + | % + | Standard error | Number + | % + | Standard error | Number + | % + | Standard error |
| 0— 4 | 16 | 2 | 13 | 8.7 | 0 | 0 | — | 2 | 13 | 8.7 |
| 5— 9 | 50 | 16 | 32 | 6.6 | 15 | 30 | 6.5 | 13 | 26 | 6.3 |
| 10—14 | 66 | 20 | 30 | 5.7 | 19 | 29 | 5.6 | 30 | 45 | 6.2 |
| 15—19 | 56 | 33 | 59 | 6.6 | 30 | 54 | 6.7 | 32 | 57 | 6.7 |
| 20—29 | 47 | 35 | 74 | 6.5 | 33 | 70 | 6.7 | 28 | 60 | 7.2 |
| 30—39 | 38 | 22 | 58 | 8.1 | 27 | 71 | 7.4 | 25 | 66 | 7.8 |
| ≥40 | 31 | 24 | 77 | 7.7 | 24 | 77 | 7.7 | 26 | 84 | 6.6 |
| Total | 304 | 152 | .. | .. | 148 | .. | .. | 156 | .. | .. |

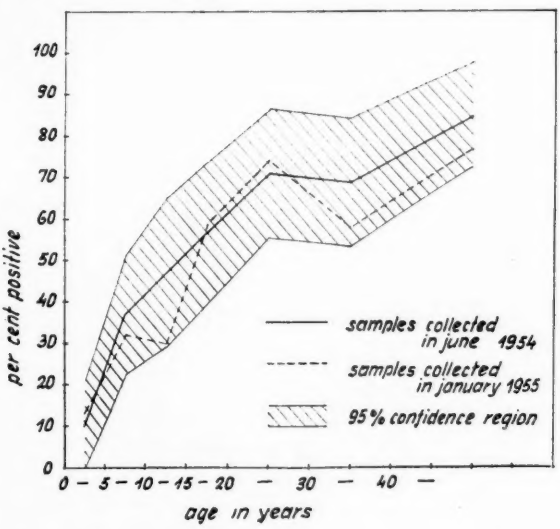


Fig. 15. — Age specific frequencies of neutralizing antibodies to type I poliomyelitis virus in the Kuhmoinen district in June 1954 and in January 1955.

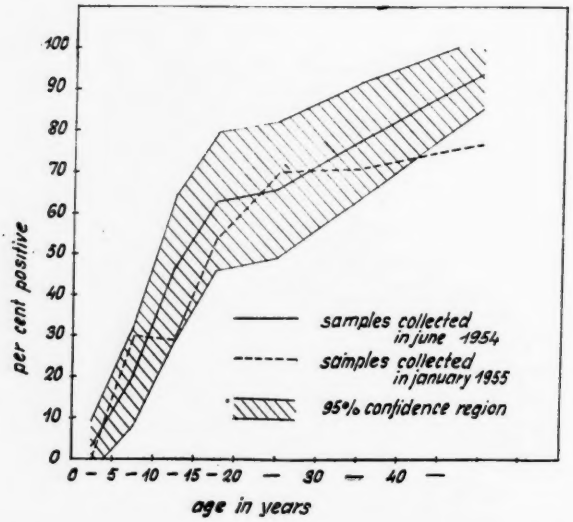


Fig. 16. — Age specific frequencies of neutralizing antibodies to type II poliomyelitis virus in the Kuhmoinen district in June 1954 and in January 1955.

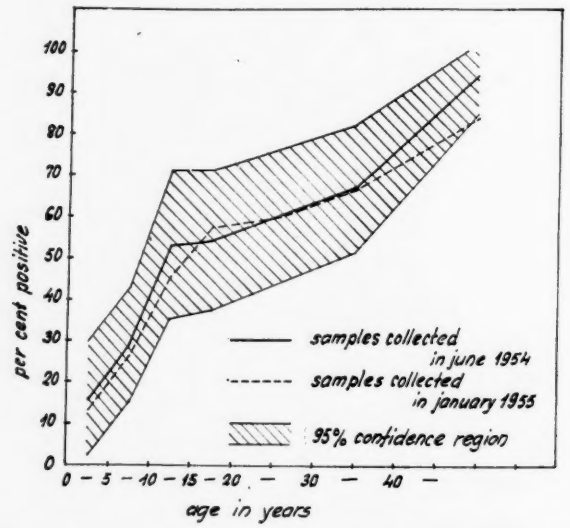


Fig. 17. — Age specific frequencies of neutralizing antibodies to type III poliomyelitis virus in the Kuhmoinen district in June 1954 and in January 1955.

differences exist between the results from the samples collected at the different times, although the proportional sampling may give a better picture of the low antibody development in the younger age groups of the entire population.

### *The Incidence of Combinations of Poliomyelitis Neutralizing Antibodies*

The combinations of the different poliomyelitis neutralizing antibodies by age groups are shown in Table 18 and in Fig. 18. This figure reveals that the percentage of sera containing three antibodies increases with age, as does also the total of all sera containing three or two antibodies.

The group of persons without poliomyelitis neutralizing antibody is remarkably large. Those investigated in this district comprised 77% of the children under 5 years, as much as 40% of the children between 5 and 9, 24% of those between 10 and 14, 11% of the persons between 15 and 30, 4% of those over 30 and 2% of those over 40.

The entire material from Kuhmoinen is approximately equally distributed between the classes of sera containing a) three, b) two, c) one and d) no antibodies whatsoever. Among the combinations of two antibodies those of type I + II and II + III are slightly more numerous (36 and 38%, respectively) than the combination I + III (26%). The sera with one antibody only show the distribution: type I in 38%, II in 25% and III in 37%.

TABLE 18

OCCURRENCE OF DIFFERENT COMBINATIONS OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE KUHMOINEN DISTRICT 1954

| Age group | Number | Three antibodies | % | Two antibodies | | | | | One antibody | | | | | No antibody | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I+II | I+III | II+III | Total | % | I | II | III | Total | % | | |
| 0— 4 | 47 | — | 0 | 1 | 1 | — | 2 | 4 | 3 | — | 6 | 9 | 19 | 36 | 77 |
| 5— 9 | 96 | 4 | 4 | 6 | 8 | 4 | 18 | 19 | 15 | 10 | 11 | 36 | 37 | 38 | 40 |
| 10—14 | 98 | 13 | 13 | 7 | 3 | 14 | 24 | 24 | 13 | 6 | 18 | 37 | 38 | 24 | 24 |
| 15—19 | 91 | 22 | 24 | 12 | 7 | 12 | 31 | 34 | 12 | 6 | 10 | 28 | 31 | 10 | 11 |
| 20—29 | 82 | 32 | 39 | 12 | 10 | 6 | 28 | 34 | 6 | 6 | 1 | 13 | 16 | 9 | 11 |
| 30—39 | 74 | 27 | 36 | 9 | 5 | 12 | 26 | 35 | 6 | 7 | 5 | 18 | 24 | 3 | 4 |
| ≥40 | 64 | 44 | 68 | 4 | 3 | 5 | 12 | 19 | 1 | 2 | 4 | 7 | 11 | 1 | 2 |
| Total | 552 | 142 | 26 | 51 | 37 | 53 | 141 | 25 | 56 | 37 | 55 | 148 | 27 | 121 | 22 |

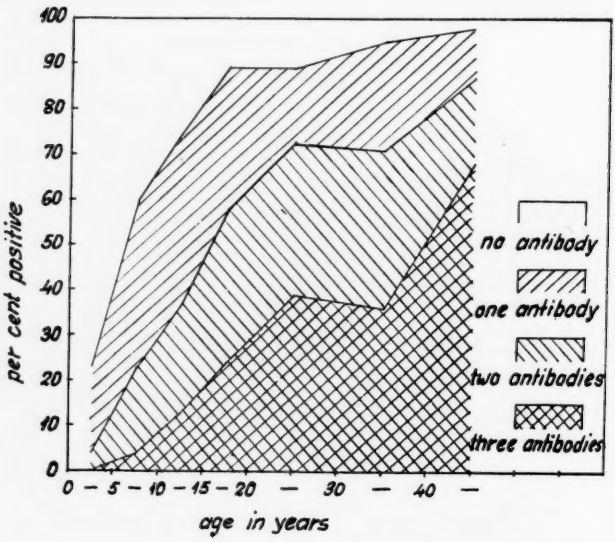


**Fig. 18. — Age specific frequencies of combinations of the neutralizing antibodies to the three types of poliomyelitis virus in the Kuhmoinen district in 1954.**

### *IIb The Joensuu District*

The district around the city of Joensuu can be seen from the map in Fig. 13, p. 65.

For the purpose of this investigation, the district of Joensuu comprises the rural communes of Ilomantsi, Kesälahti, Lieksa, Nurmes, Pielisjärvi and Kiihtelysvaara (not the town of Joensuu itself). Their aggregate area is 8,688 km² and in 1954 the population was 62,505, or 7.2 per km². The area is traversed by a railway line and also by the water system of the Pielisjoki river.

*Previous Poliomyelitis Cases:* According to reports to the State Medical Board, there were 64 poliomyelitis cases during the period 1939—53: 1 case in 1939, 17 in 1940, 5 in 1941, 1 in 1942, 2 in 1943, 3 in 1944, 1 in 1945, 5 in 1946, 2 in 1947, 1 in 1949, 4 in 1940, 2 in 1951, 1 in 1952 and 19 in 1953. All the 1953 cases came from Kiihtelysvaara commune. The age distribution of these poliomyelitis cases could not be ascertained.

The mean annual morbidity per 100,000 members of the population during the period 1939—53 was 6.8 in this area.

*The Incidence of Poliomyelitis Neutralizing Antibodies*

In the Joensuu district, the incidence of poliomyelitis neutralizing antibodies was investigated from samples of 306 persons. The results are given in Table 19. The percentual occurrence by age of the three antibodies is shown in Fig. 19.

TABLE 19

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE JOENSUU DISTRICT

| Age in years | Number examined | Sera with antibodies against | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Type I | | | Type II | | | Type III | | |
| | | Number + | % + | Standard error | Number + | % + | Standard error | Number + | % + | Standard error |
| 0— 4 | 29 | 7 | 24 | 8.0 | 4 | 14 | 6.5 | 1 | 3 | 3.2 |
| 5— 9 | 51 | 37 | 73 | 6.3 | 31 | 59 | 6.9 | 24 | 49 | 7.1 |
| 10—14 | 64 | 45 | 70 | 5.8 | 40 | 62 | 6.1 | 44 | 69 | 5.8 |
| 15—19 | 48 | 35 | 73 | 6.5 | 38 | 79 | 5.9 | 33 | 69 | 6.7 |
| 20—29 | 48 | 37 | 77 | 6.1 | 37 | 77 | 6.1 | 30 | 62 | 7.1 |
| 30—39 | 19 | 15 | 79 | 9.6 | 15 | 79 | 9.6 | 12 | 63 | 11.4 |
| ≥40 | 47 | 38 | 81 | 5.8 | 42 | 89 | 4.9 | 41 | 87 | 5.3 |
| Total | 306 | 214 | .. | .. | 207 | .. | .. | 185 | .. | .. |

The results show that neutralizing antibodies to type I occurred in 24% of the children under 5 years, reached the 50% limit at 5 years of age increasing to 73% in the 5—9 year group. This percentage remained constant up to 20 years of age, after which it increases slowly to 81% in the persons over 40.

The neutralizing antibodies to type II show an incidence of 14% in children under 5 years, and reach the 50% limit slightly later than in the case of type I. The percentage among the 10—14 year group was 59%, increasing to 79% for persons between 15 and 20 and remaining at this level up to the age of 40, after which it gradually increased to 89%.

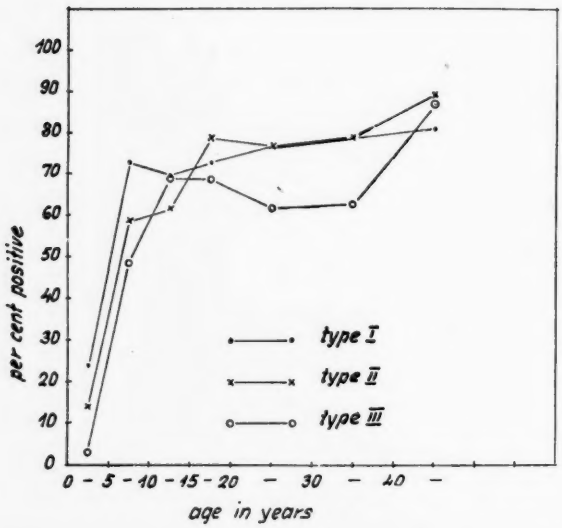


Fig. 19. — Age specific frequencies of neutralizing antibodies to the three types of poliomyelitis virus in the Joensuu district in 1954.

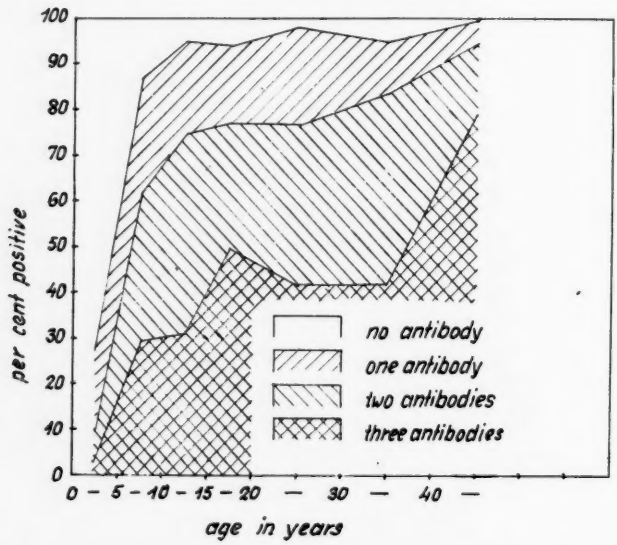


Fig. 20. — Age specific frequencies of combinations of the neutralizing antibodies to the three types of poliomyelitis virus in the Joensuu district in 1954.

Neutralizing antibodies to type III were found in a slightly smaller number of cases than antibodies to the other types. They occurred in only 3% of the children under 5 years in 49% of the children between 5 and 9 and increased to 69% in children between 10 and 14 years, whereas the percentage for persons between 20 and 40 was only 62—63%. The rate for persons over 40 was 87%.

### *The Incidence of Combinations of the Neutralizing Antibodies to the Three Types of Poliomyelitis Virus*

The different combinations of neutralizing antibodies in the various age groups, are shown in Table 20 and Fig. 20. The

TABLE 20

OCCURRENCE OF DIFFERENT COMBINATIONS OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE JOENSUU DISTRICT, 1954

| Age group | Number | Three antibodies | % | Two antibodies | | | | | One antibody | | | | | No antibody | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I+II | I+III | II+III | Total | % | I | II | III | Total | % | | |
| 0— 4 | 29 | 1 | 3 | 2 | — | — | 2 | 7 | 4 | 1 | — | 5 | 17 | 21 | 72 |
| 5— 9 | 51 | 15 | 29 | 11 | 3 | 3 | 17 | 33 | 8 | 1 | 4 | 13 | 25 | 6 | 12 |
| 10—14 | 64 | 20 | 31 | 8 | 12 | 8 | 28 | 44 | 5 | 4 | 4 | 13 | 20 | 3 | 5 |
| 15—19 | 48 | 24 | 50 | 6 | 1 | 6 | 13 | 27 | 4 | 2 | 2 | 8 | 17 | 3 | 6 |
| 20—29 | 48 | 20 | 42 | 8 | 4 | 5 | 17 | 35 | 5 | 4 | 1 | 10 | 21 | 1 | 2 |
| 30—39 | 19 | 8 | 42 | 5 | 1 | 2 | 8 | 42 | 1 | — | 1 | 2 | 11 | 1 | 5 |
| ≥40 | 44 | 35 | 79 | 3 | — | 4 | 7 | 16 | — | 1 | 1 | 2 | 5 | — | 0 |
| Total | 303 | 123 | 41 | 43 | 21 | 28 | 92 | 30 | 27 | 13 | 13 | 53 | 17 | 35 | 12 |

percentage of persons between 5 and 39 years with all three types of antibody in their sera remained fairly stationary at 30—40%, rising sharply to 79% for persons over 40. In contrast, the curve showing the percentage of persons with either two or three antibodies rises steeply to 62% for children between 5 and 9 years and then rises gradually with increasing age to 89% for persons over 40.

The group of persons with no neutralizing antibody is fairly small. 72% of the children under 5 years and only 12% of the children between 5 and 9 had no antibodies whatsoever. The percentage of children over 10 years with no antibodies is already as low as about 5%.

Among the combinations of two antibodies the combination

I + II occurred most frequently in this area, in 46%; II + III occurred in 30% and I + III in 23% of the cases.

Among the sera with one type of antibody only the most common was the antibody to type I, 51%; type II and type III were equal with 25%.

### *IIc The Kemijärvi District*

The Kemijärvi district consists of the rural communes of Kemijärvi and Sodankylä (Fig. 13, p. 65). The combined area of these communes is 15,826 km², with a population of 21,557 in 1954, giving a population density of 1.4 per km².

*Previous Poliomyelitis Cases:* During the period 1939—53 21 poliomyelitis cases were reported to the State Medical Board, i.e., 5 cases in 1940, 1 in 1941, 1 in 1944, 6 in 1945, 2 in 1948, 2 in 1949, 2 in 1950 and 2 in 1951. The age distribution of these poliomyelitis cases could not be ascertained.

The mean annual morbidity for the period 1939—53 per 100,000 members of the population in this area was 6.5.

### *The Incidence of Poliomyelitis Neutralizing Antibodies*

Blood samples from 169 persons from this area were investigated for the occurrence of neutralizing antibodies to the three types of poliomyelitis virus. The blood samples were collected in June and July, 1954.

The results are shown, according to age groups, in Table 21. In this instances, all persons over 20 years have been treated as a single group on account of their small number. Fig. 21 shows the percentual occurrence by age of the neutralizing antibodies to the different types of poliomyelitis virus.

The figure reveals that antibodies to type I occurred in 12% of the children under 5 years and that this percentage increased sharply between 5 and 9 years, passing the 50% limit at 6—7 years of age. Between school age and 20 years the incidence of antibodies to type I amounted to more than 70% and in persons over 20 years to 86%.

Antibodies to type II occurred in 6% of the children under 5 years. The percentage then increased abruptly, passing the 50%

TABLE 21

AGE SPECIFIC FREQUENCY OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE KEMIJÄRVI DISTRICT

| Age in years | Number examined | Sera with antibodies against | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Type I | | | Type II | | | Type III | | |
| | | Number + | % + | Standard error | Number + | % + | Standard error | Number + | % + | Standard error |
| 0—4 | 16 | 2 | 12 | 8.4 | 1 | 6 | 6.1 | 1 | 6 | 6.1 |
| 5—9 | 24 | 17 | 71 | 9.4 | 14 | 58 | 10.3 | 10 | 42 | 10.3 |
| 10—14 | 24 | 17 | 71 | 9.4 | 16 | 67 | 9.8 | 20 | 83 | 7.8 |
| 15—19 | 26 | 20 | 77 | 8.4 | 17 | 65 | 9.5 | 20 | 77 | 8.4 |
| ≥20 | 79 | 68 | 86 | 3.9 | 69 | 87 | 3.8 | 67 | 85 | 4.0 |
| Total | 169 | 124 | .. | .. | 117 | .. | .. | 118 | .. | .. |

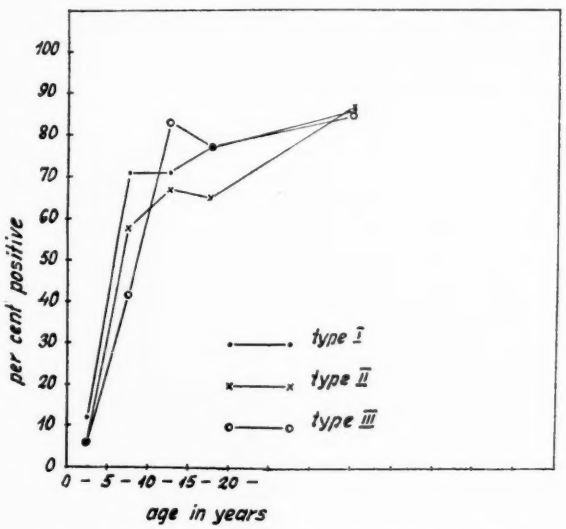


Fig. 21. — Age specific frequencies of neutralizing antibodies to the three types of poliomyelitis virus in the Kemijärvi district in 1954.

limit at 5—6 years' age. Children between 5 and 9 years showed antibodies in 58%, persons between 10 and 19 in 65—67% and persons over 20 in 87% of the cases.

Antibodies to type III, too, occurred in 6% of the children under 5 years. The percentage then increased at a slightly slower rate than that of the other types, passing the 50% limit at 8—9 years' age. In persons over 10 years the incidence already began to approach 80% and in persons over 20 it was 85%.

### *The Incidence of Combinations of the Poliomyelitis Neutralizing Antibodies*

The different combinations of neutralizing antibodies are given in absolute numbers and percentages according to the different age groups in Table 22, the same results being presented graphically

TABLE 22

OCCURRENCE OF DIFFERENT COMBINATIONS OF NEUTRALIZING ANTIBODIES TO THE THREE TYPES OF POLIOMYELITIS VIRUS IN THE KEMIJÄRVI DISTRICT 1954

| Age group | Number | Three antibodies | % | Two antibodies | | | | | One antibody | | | | | No antibody | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I+II | I+III | II+III | Total | % | I | II | III | Total | % | | |
| 0— 4 | 16 | — | 0 | — | 1 | — | 1 | 6 | 1 | 1 | — | 2 | 12 | 13 | 81 |
| 5— 9 | 24 | 7 | 29 | 4 | 2 | 1 | 7 | 29 | 4 | 2 | — | 6 | 25 | 4 | 17 |
| 10—14 | 24 | 11 | 46 | 1 | 4 | 3 | 8 | 33 | 1 | 1 | 2 | 4 | 17 | 1 | 4 |
| 15—19 | 26 | 11 | 42 | 3 | 6 | 2 | 11 | 42 | — | 1 | 1 | 2 | 8 | 2 | 8 |
| ≥20 | 79 | 55 | 69 | 8 | 2 | 6 | 16 | 20 | 3 | — | 4 | 7 | 9 | 1 | 1 |
| Total | 169 | 84 | 50 | 16 | 15 | 12 | 43 | 25 | 9 | 5 | 7 | 21 | 12 | 21 | 12 |

in Fig. 22. The material is too small to justify conclusions with regard to the different combinations. However, the high incidence, 50%, of sera with three types of antibodies is worthy of note. Neutralizing antibodies to type I occurred most frequently, both alone and in combinations.

No antibodies were found in 81% of the children under 5 years, in 17% of the children between 5 and 9, in 4—8% of the persons between 10 and 19, and in 1% of the persons over 20.

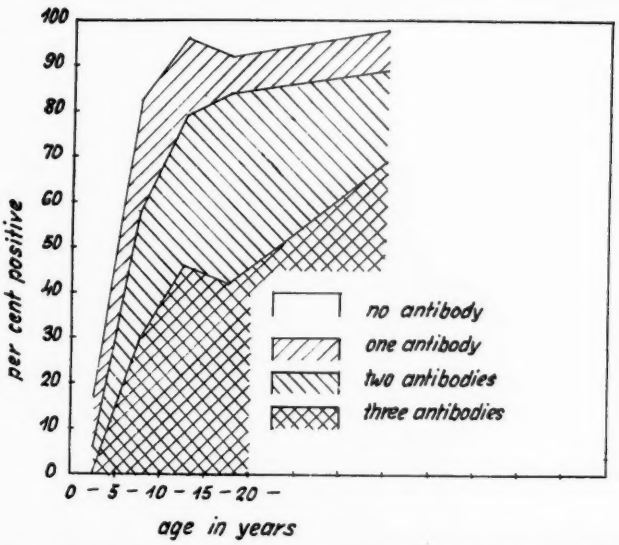


Fig. 22. — Age specific frequencies of combinations of the neutralizing antibodies to the three types of poliomyelitis virus in the Kemijärvi district in 1954.

## SUMMARY OF THE NEUTRALIZING ANTIBODY EXPERIMENTS

The incidence in Finland of neutralizing antibodies to the three types of poliomyelitis virus has been investigated in 2,111 persons drawn from four localities, 1,084 of them coming from the city of Helsinki and the remainder from the following rural areas: 552 from the Kuhmoinen district, 306 from the Joensuu district and 169 from the Kemijärvi district (cf. map on page 65). Except for some of the samples from Kuhmoinen, which were obtained in January, 1955, the blood samples were collected between January and July, 1954, before the outbreak of the poliomyelitis epidemic in August of that year.

The antibody determinations were carried out by neutralization tests in fixed roller tube tissue cultures prepared from fibroblasts of human, embryonic lung tissue. Undiluted serum and 100 $TCD_{50}$ of each virus type were used in the neutralization test.

The results showed that in all the localities investigated the percentage of persons with neutralizing antibodies to each virus type rose with increasing age. The age specific frequency of persons with the neutralizing antibodies to the three different types showed a close similarity in every district investigated.

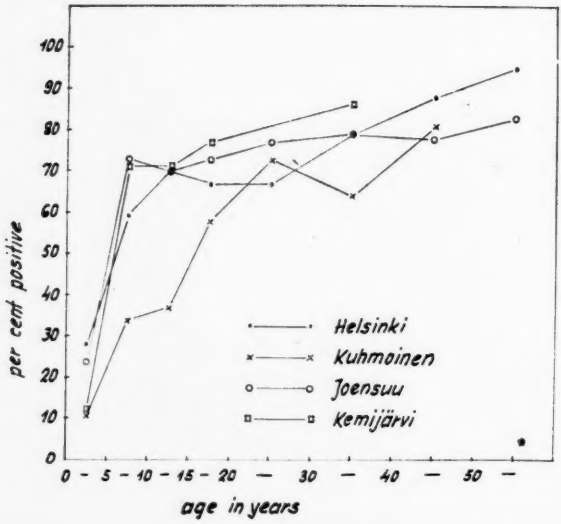


Fig. 23. — Comparison of the age specific frequencies of neutralizing antibodies to type I poliomyelitis virus in the districts investigated.

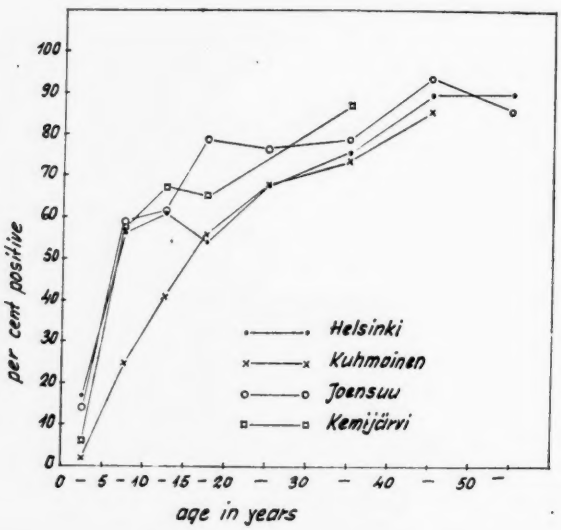


Fig. 24. — Comparison of the age specific frequencies of neutralizing antibodies to type II poliomyelitis virus in the districts investigated.

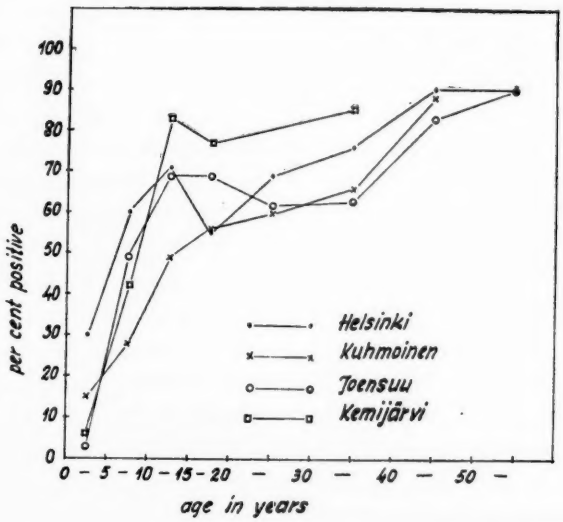


Fig. 25. — Comparison of the age specific frequencies of neutralizing antibodies to type III poliomyelitis virus in the districts investigated.

The age specific frequency of persons with poliomyelitis neutralizing antibodies varied between the different localities. (Fig. 23, 24 and 25.) The differences were not evident between the city and the rural area but between the different rural districts. In Helsinki and in the districts of Joensuu and Kemijärvi the antibody incidence was such that about 50% of the 5-year-old children possessed antibodies, 60—70% of the persons between 10 and 20 years, 70—80% of the persons between 20 and 40, and 90% of the persons over 40. In the Kuhmoinen district the antibody rate developed more slowly than elsewhere, particularly in persons under 20. In this district 11% of the children under 5 years and one third of the children between 5 and 9 showed antibodies and it was only around the 15 year level that 50% of the persons had neutralizing antibodies to each type of poliomyelitis virus. The percentage with antibodies was nearly 60% for persons between 15 and 19, 60—70% for persons between 20 and 40, and 80—90% for persons over 40.

No difference between the sexes in the development of neutralizing antibodies was observed in any one of the localities. In Helsinki the antibody rate in the different social classes was studied, as well as in the city area and suburban area. No distinct differences were observable.

The presence of only one type of neutralizing antibody to poliomyelitis virus was uncommon; as a rule the percentage was 10—20%, although the Kuhmoinen district showed 30%. The most common finding was that one person had neutralizing antibodies to all three types. About one half of the serum samples of persons under 20 years, which contained any neutralizing antibody, revealed antibodies to all three types. The percentage of such cases rose with increasing age, about 70% of all the persons over 40 having antibodies to all three types of poliomyelitis virus.

Among the combinations of neutralizing antibodies to two virus types, the combinations I + II and II + III were more common than I + III. Among the antibodies occurring alone, the antibody to type I was found to occur most frequently.

Most of the persons without neutralizing antibodies belonged to the group of children under 10 years. Persons without antibodies comprised 5—9% of all investigated persons over 10 in Helsinki and in the Joensuu and Kemijärvi districts, but in the Kuhmoinen district 24% of the children between 10 and 14 years still had no antibodies; not before the 15—19 age group did the percentage fall to 11%.

## GENERAL DISCUSSION

### *On the Age Specific Frequency of Poliomyelitis Neutralizing Antibodies*

From these studies on the neutralizing antibodies to the three types of poliomyelitis it is possible to derive a picture of the distribution of poliomyelitis infections in the districts investigated. It is evident that a high proportion of the population in these districts has acquired the infection by the time adult life is reached.

A study of the incidence of poliomyelitis neutralizing antibodies will draw attention to the low percentage of children under five years with antibodies, except for infants under one year who, as known, still have antibodies inherited from the mother. This low antibody incidence may reflect the scarcity of poliomyelitis cases in recent years.

In Helsinki neutralizing antibodies to poliomyelitis showed the greatest development in the group of children between 5 and 9, i.e., poliomyelitis infection seems to have occurred most abundantly

in this group. The same group also shows the highest number of other epidemic diseases, such as scarlet fever, in Helsinki (108).

An interesting observation when comparing the age specific frequencies of different poliomyelitis neutralizing antibodies is *the similarity between the occurrences of all three different antibodies* in every area investigated.

One explanation would be that the infecting virus strains of each of the three types occur in equal numbers and at almost the same time in every locality concerned. But in view of the virus isolations at the Municipal Bacteriological Laboratory, Helsinki, it would be surprising. From 1953 up to March 1956 201 poliomyelitis virus strains were isolated, 193 of these strains belonging to type I, 7 to type II and one to type III. Most isolations were made from poliomyelitis patients, but we have isolations from subclinical cases too, all collected during the epidemic (111). In both instances the major part consisted of infections of type I. Poliomyelitis infections from the years 1954—56 are not reflected in the neutralizing antibodies investigated in the present work. But if in the immediately preceding years the major part of clinical and subclinical infections had also been caused by one type, one would expect to find it manifested in the fluctuation of the age specific frequency curve of the particular antibody in the corresponding age group.

A more likely explanation of this phenomenon may be a heterotypic antibody response. This assumption is supported by the age specific frequency curves of simultaneous multiple antibodies. In only about 20% of the cases investigated with this method, has the presence of only one antibody in the serum been established; the simultaneous occurrence of three antibodies has been most frequent. The curve representing the total percentage of persons with either two or three antibodies is found to pass through approximately the same values in each of the investigated localities as the age specific frequency curve for any single antibody. This might be a consequence of infections with virus strains which often possessed in addition to their own antigen also antigen or at least characteristics resembling the antigen to another and often even to both other types of poliomyelitis virus. Or this might also be a consequence of anamnestic reactions. The first contacts with small doses of any poliomyelitis virus type may have given rise to small,

by our methods not measurable amounts of corresponding antibodies. In such cases subsequent infections with other types, even with one type might be able to raise neutralizing antibodies also against the first infecting type or types. This assumption is supported by Melnick's investigations (95) on complement fixing antibodies, where he shows that adults have more heterotypic antibody responses than children.

A comparison of the age specific frequencies of the poliomyelitis neutralizing antibodies in the different areas investigated shows that *the Kuhmoinen district differs from the other investigated areas*, the most significant difference obtaining in the group of persons below 20 years. The 50% antibody incident is reached about 10 years later in the Kuhmoinen district than in the other areas (at the age of 15 and 5 years, respectively). The delayed appearance of the neutralizing antibodies may be due to the circumstances that fewer poliomyelitis infections have occurred in the Kuhmoinen district. This is possible in view of the lesser occurrence of poliomyelitis cases in comparison with the other areas investigated. During the period 1939—53 the annual mean morbidity per 100,000 inhabitants was 3.5 in this district, as against 6.5—6.8 in the others.

Turner et al. (169) have computed *theoretical age distribution curves* for the neutralizing antibodies, assuming a constant rate of attack in the population under investigation. A family of such theoretical curves is shown in Fig. 26, the curves relating to the assumed annual infection of 5, 10, 20, 40 and 100% of all previously uninfected individuals of the population, one of them being based on the premise that all susceptible persons are infected within a few months (infection rate $\infty$). Turner found that the hypothetical curves constructed according to his mathematical model showed a reasonably good fit when applied to published data. Thus, the determination in merely a few age groups of the percentage of sera with antibodies would suffice to give a theoretical picture of the shape of the antibody specific frequency curve, provided that the infection rate remained constant.

In the Fig. 26 the age specific frequency curves of the neutralizing antibodies to type I have been plotted for the population of the different areas investigated in the present work together with Turner's theoretical curves. The annual infection rate in the population of the Kuhmoinen district is 5—10%, whereas

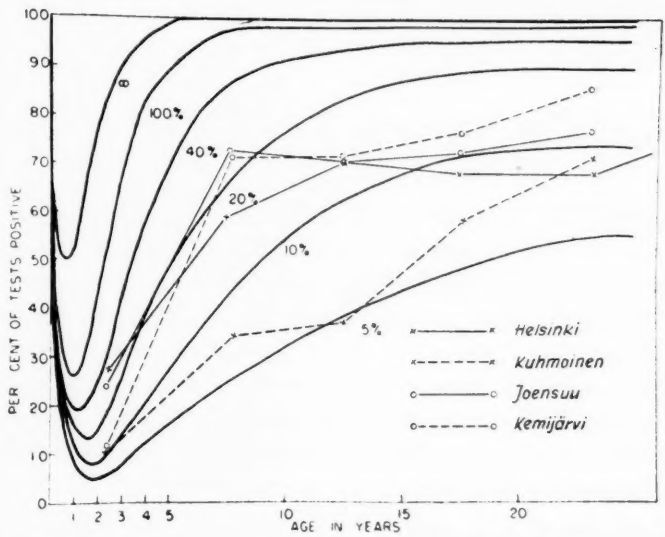


Fig. 26. — Turner's theoretical curves for poliomyelitis neutralizing antibodies and age specific frequency curves of neutralizing antibodies to type I in the districts investigated in 1954.

the curves for the other areas correspond to a rate of about 10—20%. However, the shape of these plotted curves differs from that of the theoretical curves. This deviation of the empirical figures from the theoretical ones may be caused by a number of factors.

In the first place the serum samples were not collected by means of any definite sampling method. Proportional sampling was employed with regard to the second series from the Kuhmoinen district, but no statistically significant difference was observable in comparison with the determinations from the first series, based entirely on voluntary contribution, although the appearance of antibodies showed a slight shift toward higher age in the later series. The patients (between 0 and 15 years) taken into the Aurora Hospital in Helsinki belong to all social classes, as do the blood donors of the Red Cross Blood Service (persons over 20 years). The persons between 15 and 19 years, being apprentices, were mainly from the middle classes. Thus, the Helsinki material should also give a fairly representative picture of conditions in Helsinki.

On the other hand, the differences between the actual and theoretical neutralizing antibody curves may also be caused by

unequal infection rates in the different years. This has been shown mathematically by Gard (44).

*Comparison of the Poliomyelitis Neutralizing Antibody Status in Finland with that in Other Countries*

In order that the poliomyelitis neutralizing antibody status in Finland may be compared with that in other countries, the curves for the poliomyelitis neutralizing antibodies to type I from Helsinki, Stockholm and Göteborg (Gothenburg) (114) as well as the Lansing (type II) antibody curves for Cairo, Miami and Alaska (126) are given in Fig. 27. Since all antibody types show a similar occurrence in our country, the age specific frequency curve for Helsinki of the neutralizing antibodies to type I is fully comparable with the Lansing curves mentioned.

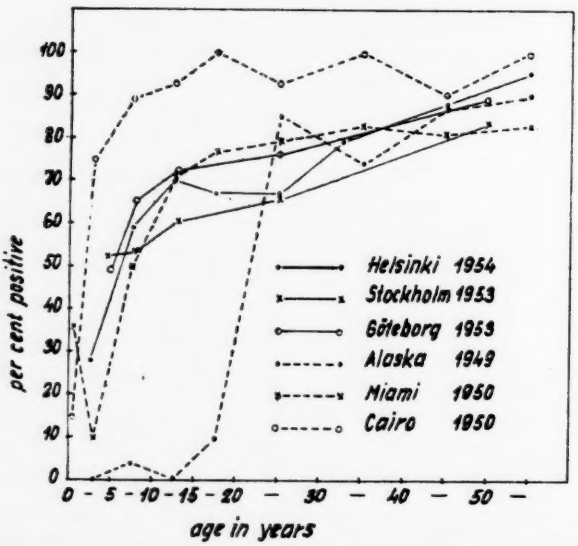


Fig. 27. — Comparison of age specific frequencies of poliomyelitis neutralizing antibodies in different populations: Stockholm, Göteborg (114), Alaska, Miami and Cairo (126).

The age specific frequency curves of the poliomyelitis neutralizing antibodies in Cairo and Alaska clearly differ from the corresponding curves from Helsinki. In Cairo a great possibility of infection is present continuously, and the empirical Lansing anti-

body distribution curve follows a similar course to the theoretical curve computed by Turner for profuse infection. In the isolated Eskimo habitations of Alaska no Lansing infection has occurred during 20 years; this is apparent from antibody occurrence.

The age specific frequency curves for type I poliomyelitis virus from Helsinki and from Sweden and for the Lansing antibodies from Miami constitute a more homogeneous group. The peculiar feature of the Stockholm curve is the high percentage (52%) of sera with neutralizing antibodies in the group of children between 3 and 5 years; this may be attributable to the recent epidemic which was caused by type I (114). In the other age groups the percentage of sera containing neutralizing antibodies after the epidemic of 1953 is somewhat lower than in Helsinki, although the sera of the material from Helsinki were collected before the onset of the 1954 epidemic. In Göteborg, where few poliomyelitis cases occurred during the 1953 epidemic in Sweden, the percentage of sera with antibodies is higher in all age groups over 5 years than in Stockholm. The age specific frequency curve for the Lansing neutralizing antibodies from Miami is not greatly different from the corresponding curves of neutralizing antibodies to type I from Helsinki and Göteborg.

*Comparison of the Age Specific Frequencies of Poliomyelitis Neutralizing Antibodies and of the Age Distribution of Clinical Cases of Poliomyelitis.*

The important question, in what proportion infection is accompanied or followed by clinical manifestations of the disease, has been studied in many populations during recent years. The fundamental investigation was carried out by Paul (126), who assumed a correlation between the local ages at which clinical poliomyelitis had been acquired and the local ages at which the Lansing neutralizing antibody had been acquired, and found »that Lansing neutralizing antibody surveys may sometimes serve as an index of general immunity to poliomyelitis in certain populations but that the index does not hold good universally.»

Paul divided his populations into primitive (A) and less primitive (B) groups; as typical representatives of those groups the investigations from Cairo and Miami are shown in Fig. 28 a).

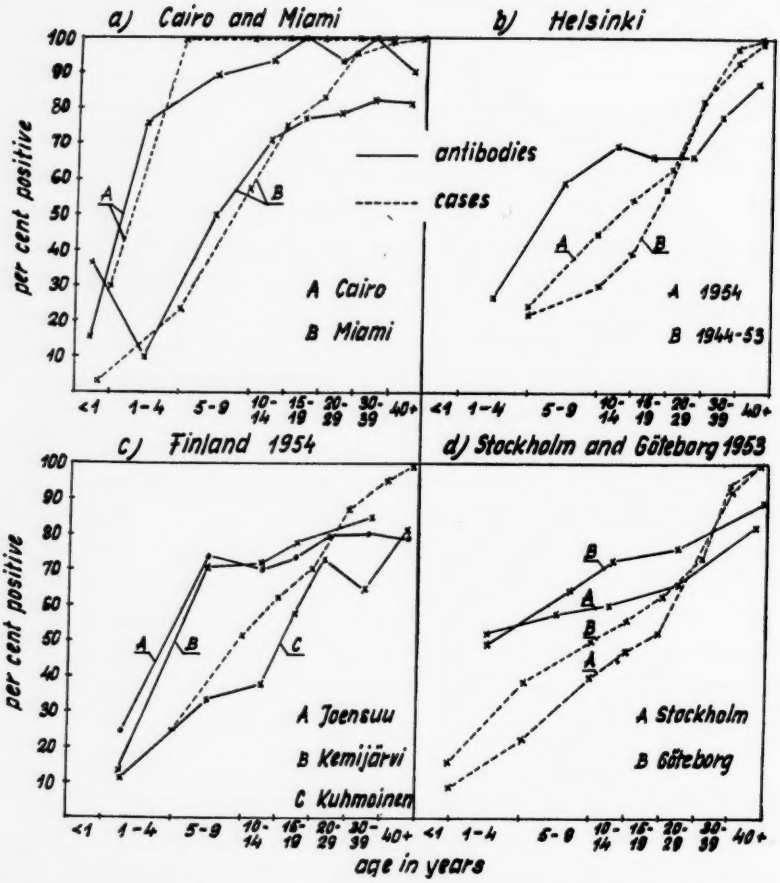


Fig. 28. — Comparison of the age specific frequencies of poliomyelitis neutralizing antibodies and the cumulative age distribution of poliomyelitis cases, in certain areas.

The development of poliomyelitis neutralizing antibodies is shown as the percentage of the sera containing antibodies among the number of investigated persons in the age group concerned, the incidence of poliomyelitis cases being shown as a cumulative per cent curve of the cases by age. The abscissa axis has been divided into larger intervals for persons under 10 years than for those over 10 years, following the procedure adopted by Paul. It will be seen from the figure that a correlation exists between the incidences of neutralizing antibodies and poliomyelitis cases in the different age groups in one and the same locality.

A similar comparison in our conditions yields the results shown in Fig. 28 b) and c). In Fig. 28 b) the percentual incidence by age group of neutralizing antibodies to type I (which is closely similar to that of the antibodies to type II and III) in Helsinki has been plotted together with the cumulative age distribution curve of the poliomyelitis cases, both from the period 1944—53 and from the epidemic year, 1954. It will be seen that the shape of the Helsinki curves resembles the type of curve from Miami considered characteristic of less primitive populations. The two poliomyelitis case distribution curves differ from each other in the age groups below 20; for instance, the 50% limit is reached at about 12—13 years' age in the 1954 epidemic year but not before the age of 17—18 years in the curve for the period 1944—53. The antibody curve passes the 50% limit already at the age of five years.

In Fig. 28 c) the cumulative per cent distribution of the paralytic poliomyelitis cases in 1954 by age for all Finland has been shown together with the curves relating to the incidence of neutralizing antibodies to type I in the rural districts investigated. The 50% limit is reached in the paralytic cases curve at slightly over 10 and in the antibody curves at 5—15 years of age, depending on the district.

The corresponding curves from Göteborg and Stockholm (114) are reproduced in Fig. 28 d). They are roughly similar to the corresponding Helsinki curves.

The Helsinki, Stockholm, and Göteborg curves all differ from the curves presented by Paul. In these districts the neutralizing antibody frequency curve reaches the 50% limit in clearly younger age groups than the cumulative age distribution curve of poliomyelitis cases. This might be attributable to the occurrence in Finland and Sweden of subclinically infecting virus strains. Another factor to be considered is that small doses of virus may give rise to a higher rate of inapparent infections than large doses (149). But it could also be explained simply by the circumstance that children are generally less seriously affected by virus infections than adults and consequently go through a subclinical or non-paralytic poliomyelitis when infected by poliomyelitis virus.

*The subclinical infection rate* is essential to an understanding of the epidemiology of poliomyelitis. To ascertain its level is of great interest but very difficult. At least two factors determine its level:

on the one hand the number and age of susceptible persons and on the other hand the virulence of the infecting virus. It is likely that the ratio varies from epidemic to epidemic and also during non-epidemic periods.

In the past, estimates of this ratio have been based on indirect methods. A ratio of 1: 100 was found by Collins (24) from a survey of the history of poliomyelitis in large groups of people. Howe (65) arrived at the figure 1: 200 from a comparison of the reported cases of an obvious disease like measles with the reported cases of poliomyelitis. Melnick (93) deduced the ratio 1: 1000 from a consideration of the amount of poliomyelitis virus in the sewage of New York City compared with the reported poliomyelitis cases in the same area.

The best method of studying this ratio would be to investigate the antibody status pre- and post-epidemically and to determine the ratio of the paralytic cases to the neutralizing antibodies developed during the epidemic in the different age groups. This is now possible, thanks to the development of new serological methods. Employing this procedure, Turner (169) found a ratio of 1: 1000, Melnick (98) 1: 175 for infants under one year, 1: 100 for children between 1 and 2 years and between 1: 60 and 1: 95 for persons over 2, while Oker-Blom (110) reached a result of 1: 230—520 in Aaland.

Gard (44) has computed the subclinical infection rate in Sweden on the basis of Turner's curves. The age specific frequency curve of the sera containing antibodies indicates an annual infection in Sweden of 10—15%; the ratio of paralytic and subclinical cases calculated from this figure was 1: 350.

The immunization percentage for Finland cannot be assessed with certainty merely from the findings in the localities investigated in this work, but if it is put at 15% the annual number of persons immunized in the entire country would be 600,000. The subclinical infection rate in 1954 would then be 1 : 1000. In earlier years the number of paralytic poliomyelitis cases was considerably less, which would further increase the denominator of the ratio, assuming that the immunization remains constant. This tends to show that immunization without clinical symptoms is more common in Finland than, for instance, in Sweden.

The same procedure, applied to the material from Helsinki, indicates an annual infection of about 15% on the basis of Turner's

curves; this is equivalent to 60,000 immunized persons 99 paralytic poliomyelitis cases occurred in the 1954 epidemic year. The subclinical infection rate in Helsinki was thus 1: 600.

A more detailed study of the subclinical infection rate has been attempted for Helsinki, where information on the age distribution of the paralytic poliomyelitis cases was available. This study has been limited to the years 1944—53, because most children were evacuated from the city during the war and the poliomyelitis figures for the war period are not reliable. The procedure for determining the annual immunization percentage of the different age groups was to smooth freely by hand the age specific frequency curve of the sera containing neutralizing antibodies and then to read from the graph the annual increments of the smoothed curve. As the size of the one year groups in the different years was known, it was then possible to compute the number of immunized persons in each one year group in the different years, assuming the immunization percentages to remain constant from year to year. From the numbers of paralytic poliomyelitis cases and of immunized persons in each one year group, the subclinical infection rates could be computed for the one-year age groups (1 to 9 years) during the 10 calender years 1944—53. The average value was found to be 1: 600 per paralytic case, its variation range in the different age groups being 1: 300—1: 700. The 95% confidence limits of the average value, based on Poisson's law are 1: 450 and 1: 750; however, the Poisson law is not strictly applicable.

*The Significance of the Poliomyelitis Neutralizing Antibody Status*

In order to be able to demonstrate the significance of the poliomyelitis neutralizing antibodies with regard to the incidence of paralytic poliomyelitis, it would be worth while to follow the incidence of paralytic poliomyelitis cases in areas with a known antibody status. It was, thus, a very fortunate coincidence that the gathering of the blood samples for this investigation immediately preceded a poliomyelitis epidemic in two of the four areas investigated, namely, in Helsinki and Kemijärvi.

In *Helsinki*, where most of the blood samples were collected before the beginning of June 1954 and even the last samples before the end of July, more poliomyelitis cases than normally began to

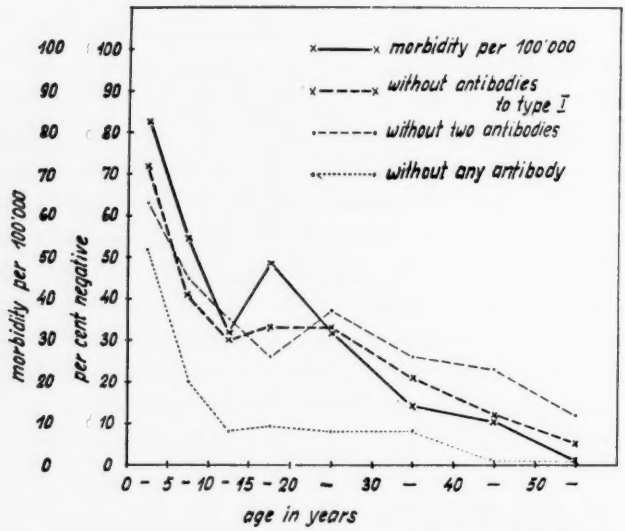


Fig. 29. — Pre-epidemical antibody status and the age distribution of paralytic poliomyelitis cases during the 1954 poliomyelitis epidemic (type I) in Helsinki.

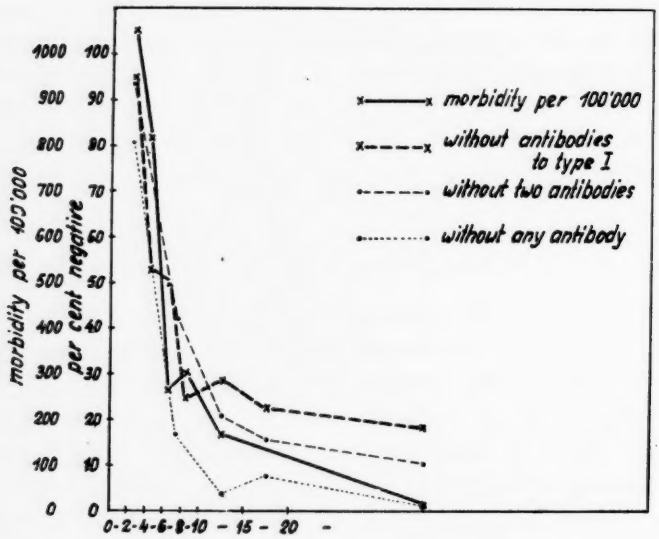


Fig. 30. — Pre-epidemical antibody status and the age distribution of paralytic poliomyelitis cases during the 1955 poliomyelitis epidemic (type I) in Kemijärvi.

occur in August of the same year (130). Virus isolations from faeces showed that the infection was caused by type I virus (111).

In Fig. 29 a comparison is made, according to age group, between the percentages of persons in whose sera no antibodies to type I, no antibodies to two types and no antibodies at all had been found and the poliomyelitis morbidity per 100,000 population in the same age group during the 1954 epidemic. The shapes of these curves correspond to each other, in particular the morbidity curve and the curves without antibodies against type I and two antibodies: a higher percentage of persons without poliomyelitis neutralizing antibodies corresponds to a higher percentage of poliomyelitis cases in the same age group; both together fall gradually with increasing age.

In *Kemijärvi*, where the blood samples had been collected in June 1954, a poliomyelitis epidemic broke out in the autumn of 1955. 21 paralytic poliomyelitis cases were reported to the State Medical Board, amounting to a morbidity of 175 per 100,000, the population of Kemijärvi being 12,000. The age distribution of paralytic poliomyelitis cases is shown in Table 23. The percentual

TABLE 23

AGE DISTRIBUTION OF PARALYTIC POLIOMYELITIS CASES DURING EPIDEMIC IN KEMIJÄRVI 1955

| Age | 0—1 | 2—3 | 4—5 | 6—7 | 8—9 | 10—14 | 15—19 | ≥20 |
|---|---|---|---|---|---|---|---|---|
| Cases ..... | — | 8 | 6 | 2 | 2 | 2 | — | 1 |
| Morbidity per 100,000 .... | — | 1053 | 816 | 264 | 303 | 170 | — | 13 |

distribution by age of persons in Kemijärvi without antibodies to type I, to two types and with no antibodies at all in their sera and the poliomyelitis morbidity per 100,000 inhabitants in the corresponding age groups are shown in Fig. 30. The curves have been plotted by 2-year age groups because 81% of the poliomyelitis cases occurred in children under 10 years.

As in Helsinki, these curves are found to concur. The absence of antibodies in persons over 10 years is less frequent than in Helsinki and so is the proportional incidence of paralytic poliomyelitis cases, too. In Helsinki as well as in Kemijärvi the proportional age distribution of the paralytic poliomyelitis cases agrees with that of the persons without poliomyelitis neutralizing antibodies. Thus,

it seems, that the poliomyelitis neutralizing antibodies have afforded protection against paralytic poliomyelitis.

It is easy to interpret the proportional age distribution of poliomyelitis cases in Helsinki and Kemijärvi on the basis of the neutralizing antibody status, but it is difficult to interpret the severity of the poliomyelitis epidemic in Kemijärvi on the same basis. The morbidity of paralytic cases in Kemijärvi was 175 and in Helsinki 25 per 100,000 inhabitants and in the most susceptible age group (0—4 years) it was about ten times higher in Kemijärvi than in Helsinki (1053—816 and 82 per 100,000 inhabitants, respectively) and yet the differences in age specific frequencies of neutralizing antibodies in the various age groups of the two regions are not very great. In the age group 0—4 in Kemijärvi there seems to be somewhat more susceptibles but on the other hand the number of susceptibles in age groups over 5 years are somewhat smaller. These differences owing to the small number of persons tested are, however, not statistically significant.

It is also difficult to interpret the comparative data of the antibody statuses and of manifest poliomyelitis cases in Helsinki and Stockholm. Unfortunately, the pre-epidemical antibody status for Stockholm is not investigated. We know the postepidemical antibody status for Stockholm and the pre-epidemical for Helsinki. It is possible that the pre-epidemical antibody status for Stockholm was low, which could explain the severity of this epidemic. It is also possible that it did not differ very greatly from the post-epidemical status. According to Melnick and Ledinko (98) the pre- and postepidemical antibody statuses differ from each other chiefly in the youngest age groups. Thus, broadly speaking both antibody statuses were of the same magnitude and the same is also true of the percentual age distribution of the paralytic poliomyelitis cases in both cities. However, the epidemics, 1953 in Stockholm and 1954 in Helsinki, were of a completely different order of magnitude, the morbidity in Stockholm being about three times that in Helsinki (72 and 25 per 100,000 inhabitants calculated from paralytic cases).

The complete absence of neutralizing antibodies seems to be an insufficient explanation for the severity of a poliomyelitis epidemic. The explanation is apparently to be found in factors determining the paralysis in the first contact with poliomyelitis virus. Possible

explanations may be that it results from differences in the virulence of the virus or in the mode of life in the different areas.

Another point of interest is the disproportion between the absolute figures for the incidence in males and females. In the areas investigated males and females displayed a similar content of neutralizing antibodies in their sera in all age groups, and the percentual age distribution of paralytic poliomyelitis cases was roughly similar (p. 38). In spite of this, and of the fact that both males and females have lived in the same circumstances and surrounded by identical viruses, their absolute figures are different. It seems, that there exist still other causes for this phenomenon.

It appears in the light of these findings that the age distribution of paralytic poliomyelitis cases follows the age distribution of the absence of poliomyelitis neutralizing antibodies. It might be possible, by ascertaining the antibody status of a locality, to predict which age groups run the greatest risk of contracting paralytic poliomyelitis. However, no advance estimate of the severity of a future epidemic can be obtained in this way, this question being obviously affected by other factors as well.

# SUMMARY AND CONCLUSIONS

*Epidemics.* — In Finland, as in most other countries during the last decades, an increase has been observed in the number of poliomyelitis cases, particularly since the 1930's, though no extraordinarily severe epidemics have occurred. The annual mean morbidity has been relatively low in Finland, in the two last decades about a third compared with the Swedish and a half of the U. S. A. morbidity. The highest morbidity, 21.5 per 100,000 inhabitants, was in 1945 and the annual mean morbidity in the 1950's has been 8 per 100,000 inhabitants calculated from all poliomyelitis cases. Poliomyelitis has had a uniform distribution in various provinces with the exception of provinces III (Ahvenanmaa) and X (Lappi), whose geographic location is exceptional. No noteworthy difference in morbidity has been observable between the urban and the rural districts.

The gradual shift in age incidence towards the older age groups, already verified in other countries, has also been observed in Finland over the last 20—30 years. Maximum attack rates remain in the 0—4 age group, but the proportion of adults among the poliomyelitis cases has increased compared with earlier years, e.g. in 1931 21 per cent of poliomyelitis cases were over 15 years of age and in 1954 38 per cent. Poliomyelitis has increased particularly in young adults; it has occurred rarely in persons over 40.

The incidence of paralytic poliomyelitis has been higher in males than in females; the morbidities for the period 1944—54 in Helsinki being 103 per 100,000 males and 65 per 100,000 females.

*Poliomyelitis Neutralizing Antibodies* were investigated in 2111 persons drawn from four localities, 1084 from the city of Helsinki and the remainder from the following rural areas: 552 from the

Kuhmoinen district, 306 from the Joensuu district and 169 from the Kemijärvi district (cf map on page 65). Except for some samples from Kuhmoinen, the blood samples were collected between January and July, 1954 before the outbreak of the poliomyelitis epidemic in August of that year.

The antibody determinations were carried out by neutralization tests with undiluted serum and 100 $TCD_{50}$ of each virus type in fixed roller fibroblast cultures prepared from human embryonic lung tissue.

The age specific frequency of poliomyelitis neutralizing antibodies showed that the percentage of persons with neutralizing antibodies increased with age. The 50% limit was reached in most of the districts investigated at 5 years of age. About 70% of the population in the districts investigated had acquired the poliomyelitis infection at 20 years of age. About 90% of the population over 40 years of age had poliomyelitis neutralizing antibodies.

Attention is called to the similarity between the age specific frequencies of the neutralizing antibodies to the three types of poliomyelitis virus in every district. This similarity is regarded as probably a consequence of heterotypic antibody response.

The percentage of persons with multiple antibodies also increased with age so that about 70% of the population concerned over 40 had neutralizing antibodies to all three types of poliomyelitis virus. Most of the persons without any poliomyelitis neutralizing antibody belonged to the group of children under 10 years. Among the combinations of antibodies to two virus types, the combinations I+II and II+III were more common than I+III. Among the antibodies occurring alone, the antibody to type I was found to occur most frequently.

There was a difference in the age specific frequencies of poliomyelitis neutralizing antibodies between the localities investigated. It was not found between the urban and rural districts, but seemed to be between districts with different histories of poliomyelitis cases. In the districts of Helsinki, Joensuu and Kemijärvi, where the annual mean morbidities during the period 1939—53 were 6.5—6.8 per 100,000 inhabitants, poliomyelitis neutralizing antibodies were acquired in younger age groups than in the Kuhmoinen district, where the corresponding morbidity was 3.5. The 50% limit to all three types was attained in other investigated

districts at about 5 years of age, but in the Kuhmoinen district at about 15 years of age.

A comparison of the age specific frequency of poliomyelitis neutralizing antibodies in the districts investigated in Finland with results from other countries showed that the age specific frequency in Finland is roughly similar to that in Sweden and in the U.S.A. in general.

*Subclinical Infection Rate.* — The subclinical infection in Finland, which seems to be relatively high, is discussed. An attempt is made to calculate the subclinical infection rate in Helsinki during recent years; among the children under 10 years of age it seems to have been about 1: 600, varying from 300 to 700.

*The Significance of the Poliomyelitis Neutralizing Antibody Status.* — The collection of the blood samples for this investigation preceded a poliomyelitis epidemic in two of the four localities investigated, Helsinki and Kemijärvi. The age distribution of the paralytic poliomyelitis cases in both districts appeared to concur with the age specific frequency of the absence of poliomyelitis neutralizing antibodies.

The idea is discussed that by ascertaining the antibody status of a district it might be possible to predict the age distribution of future paralytic poliomyelitis cases. But the age specific frequency of poliomyelitis neutralizing antibodies seems to be insufficient to predict the number of paralytic poliomyelitis cases, i.e. the severity of a future epidemic. It seems also to be insufficient to explain the different frequencies of paralytic poliomyelitis cases in males and females.

Since the antibody status of poliomyelitis neutralizing antibodies shows in which age groups the risk of contracting poliomyelitis may be high, the age specific frequency of poliomyelitis neutralizing antibodies indicates the varying vaccination requirements of different districts.

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## A STUDY OF THE OCCURRENCE OF POLIOMYELITIS AND OF NEUTRALIZING ANTIBODIES AGAINST THE THREE TYPES OF POLIOMYELITIS VIRUS IN FINLAND

BY

**KAISA LAPINLEIMU**

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